Structural Brain Differences and Motor functioning in Prenatally Methamphetamine Exposed Children in Cape Town

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DECLARATION

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ABSTRACT

Rates of methamphetamine use amongst pregnant women in South Africa is alarmingly high, rendering a large number of infants and children at risk for the adverse consequences of prenatal methamphetamine exposure (PME). Indeed, little is known about the effect of PME on brain and cognitive development in exposed children, especially in low- and middle-income settings like South Africa. The aim of the study was to contribute to the small, but growing, body of research that focuses on the brain development and motor performance of prenatally MA exposed children. The objectives were: (1) to examine the effect of PME on motor development in exposed children at the age of 8 years, compared to unexposed children of the same age; (2) to examine the effect of PME on structural brain volumes and cortical thicknesses of the brain in exposed children at the age of 8 years, compared to unexposed children; and (3) to investigate whether a correlation exists between altered brain development and motor function. Participants were 8 year old PME children (n = 17), and unexposed children (n=16) recruited from a local school and day care centre in the northern suburbs of Cape Town. PME children and unexposed controls completed two neurocognitive assessments (Beery Visual Motor Integration (VMI) test and Grooved Pegboard Test), assessing various aspects of motor function. Both groups also underwent magnetic resonance imaging (MRI). Independent sample t-tests showed that PME children scored significantly lower on measures of visual-motor integration, visual-motor coordination and fine motor development, when compared to unexposed children. Hierarchical regression analysis considering potential confounding anthropometric and socio-demographic variables and group effects, confirmed that poorer motor scores observed amongst PME children was as a result of PME. Analysis of variance (ANOVA) by group revealed that PME children had reduced cortical thickness in several brain areas that were associated with motor function.

Overall, the findings of this study contribute to the growing body of literature available on the effect of PME on brain and motor development, especially in the South African context.

OPSOMMING

Die gebruik van metamfetamien onder swanger vroue in Suid Afrika is skrikwekkend hoog, wat ‘n hoë hoeveelheid babas en kinders blootstel aan die negatiewe gevolge van prenatale metamfetamien blootstelling (PMB). Min kennis is beskikbaar oor die uitwerking van PMB op brein en kognitiewe ontwikkeling in blootgestelde kinders, veral in lae- en middel-inkomste areas soos Suid Afrika. Die hoof doel was om toe te voeg tot die groeiende literatuur rakende brein ontwikkeling en motoriese funksie in kinders met PMB. Die doelstelling was: (1) om die effek van PMB op motoriese ontwikkeling in blootgestelde kinders te ondersoek op die ouderdom van 8 jaar; (2) om die effek van PMB op strukturele brein volumes en kortikale dikte te ondersoek in blootgestelde kinders op die ouderdom van 8 jaar; (3) om te ondersoek of daar ‘n korrelasie bestaan tussen veranderinge in brein ontwikkeling en motoriese funksie. Deelnemers was 8 jarige PMB kinders (n = 17), en nie-blootgestelde kinders (n = 16) wat gewerf was vanaf ‘n plaaslike skool en dagsorg sentrum in die noordelike voorstede van Kaapstad. Beide PMB kinders en nie-blootgestelde kinders het twee neuro-kognitiewe toetse voltooi (Beery VMI toets en die Grooved Pegboard toets), wat verskeie aspekte van motoriese funksie evalueer. Beide groepe het ook magnetiese resonansbeelding ondergaan. ‘n Onafhanklike t-toets het gewys dat PMB kinders aansienlik laer presteer, vergelyking met nie-blootgestelde kinders, in toetse van visuele-motoriese integrasie, visuele-motoriese koordinasie en fyn motoriese ontwikkeling. Hiërargiese regressie-analise, wat die moontlike impak van antropometriese en sosio-demografiese veranderlikes en groep effek oorweeg het, het vasgestel dat laer motoriese tellings, onder PMB kinders, die oorsaak van PMB is. Ontleding van variansie onder groepe het gevind dat PMB kinders laer kortikale diktes in verskeie brein areas het wat verband hou met motoriese funksie. Algeheel, die bevindinge van die studie dra by tot die groeiende liggaam van

literatuur beskikbaar op die effek van PMB op brein en motoriese ontwikkeling, veral in die Suid Afrikaanse konteks.

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ABBREVIATIONS

1H-MRS	Proton Magnetic Resonance Spectroscopy
5-HT	Serotonin
ADHD	Attention-deficit/hyperactivity disorder
ATS	Amphetamine-type stimulants
BSID-II	Bayley Scales of Infant Development, second Edition
CNS	Central Nervous System
CUBIC	Cape Universities Brain Imaging Centre
DA	Dopamine
DH	Dominant Hand
GPT	Grooved Pegboard Test
HPCSA	Health Professionals Council of South Africa
IDEAL	Infant Development, Environment, and Lifestyle
IQ	Intelligence Quotient
LH	Left Hemisphere
MA	Methamphetamine
MDMA	Methylenedioxymethamphetamine
MRI	Magnetic Resonance Imaging
NDH	Non-Dominant Hand
NE	Norepinephrine
NNNS	NICU Neurobehaviour Scale
NZ	New Zealand
PDMS-2	Peabody Development Motor Scale, second Edition
PME	Prenatal Methamphetamine Exposure
PND	Postnatal Day
RH	Right Hemisphere
ROI	Region of Interest
SES	Socio-economic Status
sMRI	Structural Magnetic Resonance Imaging
UNODC	United Nations Office on Drugs and Crime

USA	United States of America
VMI	Visual Motor Integration
WHO	World Health Organisation

GLOSSARY OF TERMS

Cortical Thickness	Cortical thickness is a brain morphometric measure used to describe the combined thickness of the layers of defined regions of the cerebral cortex.
Drugs	For the purpose of this thesis, drugs refer to illicit drugs such as methamphetamine.
Fine Motor Skills	Fine motor skills refers to the coordination of small muscles in movement, such as writing or drawing.
Methamphetamine	Methamphetamine (MA) is a potent and extremely addictive stimulant type drug that affects the central nervous system upon administrating. MA works by increasing the levels of extracellular monoamine neurotransmitters in the brain. These neurotransmitters include dopamine, serotonin and norepinephrine.
Motor Function	Motor function refers to the ability to carry out complex muscle-and-nerve acts that produce movement.
MRI	Magnetic resonance imaging (MRI) is a non-invasive method that uses a magnetic field and radio waves to produce a detailed image of the brain.
PME	Prenatal Methamphetamine Exposure (PME) refers to the phenomenon where a foetus is exposed to methamphetamine during pregnancy.

Substance	For the purpose of this thesis, substance refer to substances such as alcohol or cigarettes.
Visual-Motor Integration	Visual motor integration refers to the ability to coordinate fine motor skills with visual-spatial perception. This enables an individual to build a puzzle, draw and copy geometric forms or arrange blocks in a pattern to resemble a certain form.
Visual Spatial Perception	The way a space is perceived, it can affect both fine and gross motor skills.

CHAPTER 1

INTRODUCTION

1.1 Background

With more than 35 million users worldwide, methamphetamine (MA) (also commonly known as Tik, Meth, Ice, Chalk, and Crystal) is a serious and hugely burdensome public health concern (United Nations Office on Drugs and Crime, 2014). The increased use of MA, amongst pregnant women is not only detrimental to the mother, but prenatal MA exposure (PME) has long-lasting physical, neurodevelopmental and cognitive effects on the developing foetus (Chang et al., 2004; Cloak et al., 2009; Nguyen et al., 2010).

A steadily growing body of literature demonstrates that PME children experience differences in structural brain volumes and cortical thicknesses, when compared to unexposed children (Berman, O'Neill, Fears, Bartzokis, & London, 2008; Chang et al., 2004; Diaz et al., 2014; Roos et al., 2014; Sowell et al., 2010). Furthermore, damage to the brain, caused by PME, may interfere with long-term cognitive abilities such as motor function and visual-motor integration (Chang et al., 2004).

1.1.1. Global use of methamphetamine

MA was developed in the early 20th century and forms part of the Amphetamine group of drugs (World Health Organization, 2004). Amphetamine-type stimulants (ATS) have grown rapidly in popularity over the last few years. ATS includes MA, amphetamine, methylenedioxymethamphetamine (MDMA), and other designer drugs. Increasing in popularity, ATS has become the most sought-after psycho-stimulant in the world (Chomchai & Chomchai, 2015; UNODC, 2014).

MA can be administered by smoking, snorting, injecting as well as oral ingestion. The preferred method of use varies by geographical area, although smoking this drug seems to be the preferred method of use (Volkow, 2013). By injecting or smoking MA, the drug gets absorbed into the bloodstream more rapidly. MA comes in the form of a white/off-white, odourless crystal that can easily dissolve in water or alcohol (Volkow, 2013).

1.1.2. Methamphetamine use in the Western Cape

MA is used widely across South Africa. However, MA abuse is disproportionately high in the Western Cape compared to other South African provinces. It is estimated that 7% of the adult population, residing in the Western Cape, use MA frequently, with the highest rate in Cape Town (Jones et al., 2011; Piper et al., 2011). MA is the most popular drug in the Western Cape, with at least 36% of all individuals admitted for drug rehabilitation in 2009 indicating use of MA (Plüddemann, Parry, Bhana, Dada, & Fourie, 2010).

Within the Western Cape there are many communities with a low socio-economic status. These communities experience high levels of unemployment, poverty, gang-related violence, and alcohol and drug abuse. Poverty and unemployment has been found to exacerbate substance abuse and is often seen as a coping strategy for people residing in low-income communities (Onah, Field, Heyningen, & Honikman, 2016).

1.1.3. Methamphetamine use amongst pregnant women

In the United States of America (USA), the prevalence of MA abuse amongst pregnant females has increased from 8% in 1994 to 24% in 2006 (Terplan, Smith, Kozloski, & Pollack,

2009). Similarly in South Africa in 2006, Plüddemann et al. (2008) found that more than 90% of female MA users are of childbearing age.

Studies on substance abuse trends amongst MA users in the Western Cape have indicated that MA is most commonly used amongst men, although women are especially susceptible to the multiple dangerous risks associated with the use of MA (Jones et al., 2011).

In a South African study, conducted among local mixed race individuals in the Western Cape province, Jones et al. (2011) found that amid 356 non-pregnant females, in their 20's, 238 of them used MA. This accounted for more than 66% of females, around the age of 20, of mixed race, abusing MA. From the sample group, 24 out of 26 pregnant females (92%) abused MA (Jones et al., 2011). Similar findings were reported in a study conducted by Everett-Murphy et al. (2010) in Cape Town. The authors found 78% of pregnant women, who smoked tobacco, also used MA on a frequent basis.

This increased risk is often considered against the backdrop of South African history and socio-economic and cultural factors. Indeed, women living in historically disadvantaged communities are particularly vulnerable to MA use and associated consequences (Kapp, 2008; Morris & Parry, 2006; Wechsberg, Luseno, Riehman, Browne, & Parry, 2008). For example, MA frequently causes users to engage in risky sexual behaviour. These behaviours include sexual engagement with multiple partners, unprotected sex as well as working in the sex trade. These high-risk sexual behaviours often lead to unplanned pregnancies, and many continue, or increase, the use of MA during pregnancy (Simbayi et al., 2006; Wechsberg et al., 2008).

The risks and consequences associated with the use of MA, are amplified during pregnancy (Jones et al., 2011). An increase in MA use is often the result of partner conflict or abuse (Moylan, Jones, Haug, Kissin, & Svikis, 2001), psychiatric comorbidities (Tuten, Jones,

Tran, & Svikis, 2004), lack of social support (Roberts & Pies, 2011), as well as the lack of supporting health facilities (Semple, Zians, Strathdee, & Patterson, 2007; Smith et al., 2006).

1.1.4. Methamphetamine mechanism of effect in pregnant mother and foetus

At a cellular level, MA works by increasing the release of extracellular monoamine neurotransmitters in the brain. These neurotransmitters include dopamine (DA), serotonin (5-HT) and norepinephrine (NE) (Kish, 2008). MA acts as a substrate for the plasma membrane DA and NE transporters; therefore MA is transported into cells. MA causes a substantial increase in synaptic levels of catecholamine neurotransmitters. This is caused by MA's ability to reverse transport and cause plasma membrane transporters to release neurotransmitter molecules into the synapse instead of performing their normal re-uptake function (Sulzer, Sonders, Poulsen, & Galli, 2005).

The physiological effects of MA use manifests in various ways, and often causes an increase in arousal, wakefulness, alertness, energy, motor and speech activity, self-confidence, and concentration (World Health Organization, 2004). It also induces a feeling of euphoria and overall well-being. MA abuse is also associated with a decrease in appetite, restlessness, mild confusion, tremors, panic attacks and induced episodes of psychosis (World Health Organization, 2004).

The physical effects MA induces in the mother has harmful effects on the foetal environment which can interfere with the development of the child (Khoradmehr et al., 2015). For example, in their study, Khoradmehr et al. (2015) demonstrated that MA can cause vasoconstriction. Vasoconstriction is the process where blood vessels constrict, which causes a decrease in blood flow. A decrease in utero-placental blood flow can lead to foetal hypoxia and ultimately foetal death. Malnourishment amongst MA users is common, due to MA being

an appetite suppressant. Malnourishment during pregnancy has harmful effects on the development and growth of the foetus (Khoradmehr et al., 2015).

When MA is used during pregnancy, it has the ability to cross the placenta, reach the foetus as well as cross the blood-brain barrier to exert its effects on the developing foetal brain. The human foetus is highly susceptible to the effects of MA, since its blood-brain barrier is highly penetrable, more than those of children and adults. The human foetus is not yet capable of successfully detoxifying and metabolising the drug, therefore making them more susceptible to the damaging effects of MA (Khoradmehr et al., 2015).

1.1.5. Effects of methamphetamine on brain development and motor function

Previous evidence suggests that children who have been prenatally exposed to MA may experience certain developmental problems compared to unexposed children. These restrictions become evident when you compare structural brain imaging data and cognitive tests of PME children to unexposed children. Structural differences have been associated with cognitive deficits in the following domains: sustain attention, verbal and memory tasks, motor tasks as well as visual motor integration (Chang et al., 2004; Cloak et al., 2009).

Information available on the effect of PME on the motor development of children is limited. Studies found motor impairments in infants (under the age of 1), visual-motor impairment in children between the ages of 3-5 (Cernerud et al., 1996; Chang et al., 2009; LaGasse et al., 2011; Piper et al., 2011; Wouldes et al., 2014) and some studies grouping younger children together with adolescents found fine motor impairments in PME children (Chang et al., 2004; Sowell et al., 2010).

1.2. Motivation for this study

MA use is disproportionately high in the Western Cape. Based on available evidence, it is evident that a large amount of pregnant women residing in the Western Cape province, will use MA during pregnancy, which present risks for both the mother and child (Roos, Jones, Howells, Stein, & Donald, 2014). The effects of PME on motor function and brain development in early childhood (8-9 years of age), is poorly understood and more evidence is needed to facilitate the development of appropriate interventions.

At the time of this writing and based on my review of literature, no studies on the effect of PME on the structural brain development and cognitive functioning among children 8-9 years has been published. While the development of fine motor functioning is ongoing and increases progressively in complexity from infancy up until the ages of 13-14 years, it is possible to detect abnormalities among children at age 8 and 9 as these functions are expected to be developed at the age of 6 years (Shonkoff & Phillips, 2000) and is continually refined into adolescents.

Considering the age of the child is critical when investigating the effect of PME in children, due to developmental trajectories. There may be differences in brain development over time of PME children, compared to unexposed children, due to aberrant brain development and reduced brain plasticity (Roos et al., 2014; Sowell et al., 2010).

1.3.Research question

To what extend do children who have been exposed to methamphetamine prenatally differ from healthy children in terms of structural brain development and motor functioning?

1.4. Research aim and objectives

The aim of the study was to contribute to the small, but growing, body of research that focuses on the brain development and motor performance of prenatally MA exposed children. The research objectives were threefold:

(i) To determine if there was a difference in motor function between PME and unexposed children by comparing their results of the Beery Visual-Motor Integration (VMI) test and the Grooved Pegboard Test (GPT).

(ii) To determine whether a difference exists in structural brain volumes and cortical thickness in the motor centres and associated areas of the brain when comparing structural brain data of MA exposed 8-9 year old children to unexposed children.

(iii) To investigate whether a correlation exists between the structural brain data and the results of the Beery VMI test and the GPT.

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1.4. Overview of the thesis

Chapter 1 provides an introduction to the study. The motivation, research aim and objectives of the study are outlined. The chapter concludes with an overview of the thesis.

Chapter 2 provides relevant literature pertaining to the study. This includes literature on studies that has been done on the effect of PME on the physical, brain and motor development of children.

Chapter 3 contains the research methodology that was used to obtain and analyse the data. This chapter includes the research design, information about the participants, the

procedure that was followed, as well as a discussion of the measures that were used. Data analyses, as well as matters concerning ethics, are also discussed.

Chapter 4 contains the cognitive and structural brain data results of the present study.

Chapter 5 contains the discussion of the results, limitations and implications.

CHAPTER 2

LITERATURE REVIEW

In this chapter I review the relevant literature pertaining to prenatal MA exposure (PME) on the developing foetus, and the structural and cognitive abnormalities that may present as a consequence of prenatal exposure. Firstly, I provide the literature search strategy that was used to locate relevant literature. Secondly, I will discuss the effect of PME in animal models, followed by a discussion of the effect of PME in humans (physical -, neuro- and cognitive development).

2.1. Literature search strategy

I began my search for relevant literature, on PME, by consulting academic databases such as: MEDLINE, ProQuest Medical Library, ProQuest Social Science Journals, PsycARTICLES, PubMed, ScienceDirect and Scopus. My search string consisted of relevant terms and phrases such as, “prenatal AND methamphetamine AND exposure AND physical development”, “prenatal AND methamphetamine AND exposure effect AND developing brain OR brain structures OR brain volumes OR brain functioning”, and “prenatal AND methamphetamine AND exposure AND cognitive development OR cognitive functioning OR motor development. Given the limited literature available on the effect of PME, I also referred to the bibliographies of all the academic articles I could find. Therefore, I investigated all articles in the bibliographies that referred to the effect of PME on cognitive, physical or brain development in humans and animals.

2.2. Prenatal methamphetamine exposure in animal models

2.2.1. Prenatal methamphetamine exposure effect on physical and brain development

Numerous animal studies have been conducted amongst pregnant rodents to determine the effect of PME on brain development and cognitive functioning (Heller, Bubula, Freeney, & Won, 2001; Khoradmehr et al., 2015; Mirjalili, Kalantar, Lahijani, Sheikhha, & Talebi, 2013; Moore et al., 2011; Siegel, Crayton, & Raber, 2010; Šlamberová et al., 2014; Won, Bubula, Mccoy, & Heller, 2001). These studies have repeatedly shown that administering pregnant rodents with different doses of MA during different time points in their 17-20 day gestational period, have adverse outcomes on the development of the rodent foetus (Heller, Bubula, Freeney, & Won, 2001; Khoradmehr et al., 2015; Mirjalili, Kalantar, Lahijani, Sheikhha, & Talebi, 2013; Moore et al., 2011; Siegel, Crayton, & Raber, 2010; Šlamberová et al., 2014; Won, Bubula, Mccoy, & Heller, 2001). In a study by Khoradmehr et al. (2015), the effects of a 10mg/kg/day dose of MA on pregnant mice and their offspring was evaluated. The authors found that this daily dose of MA resulted in a reduction in appetite which perpetuated weight loss. Other physical abnormalities included, a smaller head and placenta circumference, haemorrhaging (ruptured blood vessel in the brain) in the cerebral cortex sub-ependymal zone, as well as a decreased crown-rump length (Khoradmehr et al., 2015).

Animal studies also propose that MA abuse during the first and third trimester can cause long-term consequences in dopamine (DA) and serotonin systems and effect learning and social development (Khoradmehr et al., 2015). For example, a study by Heller et al. (2001) found that MA increases DA levels in the foetal rodent brain. These increases were predominantly in the striatum (an area of the brain responsible for multiple aspects of

cognition, motor, action planning, and is an important part of the reward system) and the frontal cortex (an area of the brain that together with other regions are involved in motor function, problem solving, and memory) (Heller, Bubula, Freeney, & Won, 2001; Won, Bubula, Mccoy, & Heller, 2001). A disruption in the dopaminergic system, in the early stages of development, can influence certain developmental functions in adulthood e.g. locomotion (Heller et al., 2001).

2.2.2. Prenatal methamphetamine exposure effect on cognitive functioning

A study by Šlamberová, Pometlová and Charousová (2014), found that PME rats perform worse in tests for balance and sensory-motor coordination (Šlamberová et al., 2014). These poor performances might be explained by impaired sensory inputs and delayed development of control of locomotion that is caused by PME. The study also showed that PME impairs postural reactions and movements (Šlamberová et al., 2014). One might speculate that the poorer scores observed in motor function might be the result of alterations in the striatum and the frontal cortex.

Additionally, some evidence suggests that PME impacts memory functioning (Moore et al., 2011; Šlamberová et al., 2014). In their study Šlamberová et al. (2014) found that PME rats performed worse on tests for object recognition when presented with old objects. Their data suggests that PME impairs non-spatial memory more than spatial memory (Šlamberová et al., 2014). Siegel et al. (2010) also found that PME rats experienced impaired object recognition later in life (Siegel et al., 2010).

2.3. Effect of prenatal methamphetamine exposure on the developing human foetus and child

In-utero exposure of MA seems to effect the growth, physical-, neuro-, and cognitive development of a human foetus (Nguyen et al., 2010; Chang et al., 2004; Cloak et al., 2009).

2.3.1. Physiological effects of prenatal methamphetamine exposure

It has been suggested that MA affects the developing foetus via direct placental transference (i.e. where the drug is transferred from the mother to foetus via the placenta). Foetal environmental changes, caused by the use of MA, can also be harmful to the foetus and the development process. For example, malnourishment in women who abuse MA is common, due to MA being an appetite suppressant. A lack of nutrition, during pregnancy, has harmful effects on the growth and development of the foetus (Khoradmehr et al., 2015). In a recent study Khoradmehr et al. (2015), demonstrated that MA has a vasoconstrictive effects. Vasoconstriction, the constriction of blood vessels, in pregnant women can cause a decrease in utero-placental blood flow which can lead to foetal hypoxia (low levels of oxygen in the foetus). Foetal hypoxia significantly imposes on normal brain development and growth of a foetus (Chang et al., 2004; Khoradmehr et al., 2015).

In-utero exposure to MA can lead to foetal death by causing calcification and morphological damage to the placenta (Khoradmehr et al., 2015). Other effects, commonly associated with PME, includes a decline in the birth weight, growth retardation, a smaller head circumference, cardiac anomalies, premature birth, cerebral haemorrhage, as well as cleft palate (Smith & Santos, 2016). PME can also effect the liver and cardiovascular system of the foetus (Khoradmehr et al., 2015).

2.3.2. Structural brain differences in prenatal methamphetamine exposure

Numerous brain development processes occurs in-utero. These processes include cell proliferation (i.e. cell division), cell migration (process by which cells move from one location to the next), cell differentiation (process where a cell becomes specialized in a specific function) and myelination (the production of the myelin sheath). Given that so many crucial brain development processes are occurring in-utero, there are multiple opportunities for PME to affect the neurodevelopment of a foetus and ultimately have implications on cognitive performance (Diaz et al., 2014).

2.3.2.1. Frontal structures

A few studies on the effect of PME, on the development of the brain, have found that PME can cause volumetric and cortical thickness alteration in frontal brain structures. These frontal structures include: anterior cingulate, posterior cingulate, frontal gyrus, and parsopercularis (Roos et al., 2014; Sowell et al., 2010). The frontal lobe takes up approximately one-third of the brain's cortical surface. This area of the brain is involved directly and indirectly as part of brain networks with a wide range of human functions. These functions involve simple motor function (fine and gross), complex motor function, automatic motor skill, attention, judgement, problem solving, emotional regulation and impulse control (Scott & Schoenberg, 2010).

A study by Sowell et al. (2010) found structural differences in the frontal areas of PME children. They collected Magnetic Resonance Imaging (MRI) scans on 61 children between the ages of 5 and 15 years old. The study included children who were affected by MA (N=21), MA and alcohol (N=18), only alcohol (N=13), as well as unexposed controls (N=27). The authors included children exposed to both substances due to the fact that mothers who abuse MA often abuse alcohol as well, which is a known teratogen. They found

that children exposed to MA and MA/alcohol had volumetric increases in the anterior cingulate, as well as volumetric decreases in the inferior frontal gyrus. This study was the first study to report volumetric alterations in the cingulate cortices of PME children (Sowell et al., 2010). The anterior cingulate cortex forms part of the attentional network, which may be deficient in PME children. The attentional network is involved with the monitoring of control, decision making and the connection of sensory input with executive brain centres in generating motor output (Chang et al., 2004). The anterior cingulate is also strongly interrelated with the medial temporal lobe, which also demonstrated an increase in volume (Sowell et al., 2010). Recent studies suggest that the inferior frontal gyrus plays an important role in action observation and imitation (Molnar-Szakacs, Lacoboni, Koski, & Mazziotta, 2005). One can argue that alterations in the anterior cingulate cortex, which forms part of the attentional network, can cause poor motor function in PME children. The difficulties PME children experience in motor performance will be discussed in more depth later on (see section 2.3.4). The authors also found that PME children showed more prominent volumetric increases in limbic structures such as the posterior cingulate (Sowell et al., 2010). Studies suggest that the posterior cingulate plays an important role in cognitive functioning, although there is no consensus on its exact role (Leech & Sharp, 2014). However, a lesion study (a study on brain abnormalities) suggests that the posterior cingulate is linked to spatial memory (Maddock, Garrett, & Buonocore, 2001).

A study, conducted by Roos et al. (2014), investigated potential changes in brain volumes and cortical thickness in the presence of PME. The study included 18 PME children and 18 unexposed children between the ages of 6 and 7 years. All children were recruited from a local school and care centre in the Cape Town area. In the frontal areas they found that PME children had reduced cortical thickness in the parsopercularis, which forms part of the inferior frontal gyrus (Molnar-Szakacs et al., 2005; Roos et al., 2014).

2.3.2.2. Temporal structures

The temporal cortex is located below the frontal lobe. This area of the brain is mostly involved with language, hearing, sound and some aspects of memory and emotions (Banich & Compton, 2011; Robinson, 2011). A study by Sowell et al. (2010) investigated the potential changes in brain volumes in the presence of PME. The authors found that PME was associated with volumetric increases in the inferior and medial (centre or middle area) temporal cortices (Sowell et al., 2010).

2.3.2.3. Parietal structures

Studies on the effect of PME, on the developing brain, has found that PME can lead to both volumetric and cortical thickness alterations in parietal structures. The inferior parietal-, as well as the precuneus areas are both areas associated with alteration in the presence of PME (Roos et al., 2014). The parietal structures play a fundamental role in the integration of information from different sensory modalities, as well as integrating information that is stored in memory with information from the sensory world (Banich & Compton, 2011).

A local study by Roos et al. (2014) on the effect of PME on brain volumes and cortical thickness also found brain changes in PME children's parietal areas. The authors found a reduction in cortical thickness in inferior parietal areas, as well as in the precuneus areas (Roos et al., 2014). Studies have showed that the precuneus is involved with a wide spectrum of tasks, including self-processing operations, episodic memory retrieval, and visuo-spatial imagery (Cavanna & Trimble, 2006).

2.3.2.4. Subcortical structures

Studies on the effect of PME, on brain development, have shown that subcortical structures seem to be most susceptible to the harmful effects of PME. Studies have found

volumetric alterations in numerous subcortical structures, which includes: caudate bilaterally, putamen bilaterally, globus pallidus, hippocampus, striatum and the thalamus (Chang et al., 2004; Roos et al., 2014; Sowell et al., 2010).

Chang et al. (2004) conducted a study to examine the effect of PME on volumetric development of subcortical structures and related cognitive deficits in children. They hypothesised that dopamine-rich areas of the brain, such as the striatum, will be most vulnerable for deficits in exposed children, since MA affects the dopamine system in adult MA users. The study compared the overall brain volumes and regional brain structures in 13 PME children and 15 unexposed children by the use of MRI scans. Smaller brain volumes were observed in the basal ganglia (Chang et al., 2004). The basal ganglia consist of a variety of subcortical cell groups which are involved in communicating with motor regions in the cortex via the thalamus. The two major structures of the basal ganglia are the putamen and the caudate nucleas, also referred to as the striatum, with the adjacent globus pallidus (Lanciego, Luquin, & Obeso, 2012; Zillmer, Spiers, & Culbertson, 2008). The study found compared to controls reduced volumes in both striatal areas: caudate bilaterally (-13%) and putamen bilaterally (-17.7%), as well as reduced volume in the globus pallidus (left: -27%, right: -30%) (Chang et al., 2004). The basal ganglia plays a fundamental role in motor function, since it is involved in the control of higher order movement, particularly in starting or initiating movement (Zillmer et al., 2008). Additional to finding reduced volumes in the basal ganglia, Chang et al. (2004) also found reduced volumes in the hippocampus (left: -19%, right: -20%), a structure involved in learning, memory and emotion (Chang et al., 2004; Zillmer et al., 2008).

The study by Sowell et al. (2010) supported the findings of Chang et al. (2004) by finding similar alterations in the basal ganglia and other subcortical structures. Their findings,

similar to those of Chang et al. (2004) showed that both groups of children exposed to MA, demonstrated extensive volumetric reduction in the striatum and thalamus (Sowell et al., 2010). The thalamus plays a crucial role in enabling movement. Pathways that enable communication between the basal ganglia and the motor regions in the cortex, to generate movement, run through the thalamus (Sommer, 2003). Their findings suggest that the striatal and limbic structures are most vulnerable in the case of PME, which is also the areas of neurotoxicity in adult MA abusers (Sowell et al., 2010).

Roos et al. (2014) also found significant structural differences in PME children in striatal areas. They found that the volume in the left putamen was significantly increased. Berman et al. (2008) found a reduction in striatal volumes in PME children (Berman, O'Neill, Fears, Bartzokis, & London, 2008). Although the direction of finding are contradictory amongst studies e.g. Chang et al. (2004) and Sowell et al. (2010) also found decreased volume in the putamen, the finding of altered striatal volume is consistent with findings on adult MA exposure (Chang, Alicata, Ernst, & Volkow, 2007).

The findings, of the above studies, on the effect of PME on the developing human brain are summarised in Table 1. The table categorizes the findings according to the age of the children, the region of the brain that was affected, as well as the cognitive implications of brain alterations.

Table 1: Structural brain differences in prenatal methamphetamine exposure

Author	Age	Frontal	Temporal	Parietal	Subcortical Structures	Correlation with Neuropsychological measures
Chang et al., 2004	3-16		↓Vol hippocampus		↓Vol globus pallidus, ↓Vol putamen, ↓Vol caudate	Decline in Verbal Memory, VMI, Attention, long-term spatial memory performance.
Chang et al., 2009	3-4				↓ MI in thalamus	Decline in performance in VMI tasks.
Sowell et al., 2010	5-15	↑ Vol posterior cingulate, ↑ Vol anterior cingulate, ↑ Vol inferior frontal gyrus,	↑ Vol inferior and medial		↓ Vol striatum, ↓ Vol thalamus,	
Roos et al., (2014)	6	↓ CT of pars opercularis		↓ CT of precuneus areas; ↓ CT of inferior parietal areas	↑ Vol in Left Putamen	

Note: sMRI = Structural Magnetic Resonance Imaging; VMI = Visual Motor Integration; MRS = Magnetic Resonance Spectroscopy; Vol = Volume; MRI = Magnetic Resonance Imaging; CT = Cortical Thickness

2.3.3. Gender differences in prenatal methamphetamine exposure

Evidence suggests that the damages acquired by PME are sex-dependent. Based on the findings of animal studies it has been suggested that males are more vulnerable to the detrimental effects of MA exposure than females (Gomes-da-silva, De Miguel, Fernandez-Ruiz, Summavielle, & Tavares, 2004). However, findings may vary by developmental stage and brain region.

An animal study investigating neonatal and prepubescent estrogen levels in PME mice, suggests that estrogen in female mice serves as a partial neuroprotective factor against the harmful effects of MA (Dluzen & McDermott, 2002). Therefore, it is argued that during certain stages of development, males are more vulnerable to the harmful effects of MA than females.

A study by Gomez-Da-Silva et al. (2004) investigated the sex-dependent effects of MA on new born mice. They administered postnatal day (PND) 1 mice with 10mg/kg of MA daily. They administered MA to the mice up until the day they were euthanized. Mice were euthanized on PND7, PND14, and PND30. They found that neonatal MA-exposure caused an increase in levels of NE in the substantia nigra of PND30 rats (male and female), however the same variation was evident only in male rats by PND14. They also observed that MA-exposure caused an increase in NE levels in the caudate-putamen of PND7 males and PND14 females (Gomes-da-silva et al., 2004).

A study by Roos et al. (2014), investigated the effect of PME on 6 year old children. The authors found that PME boys had an increased right diencephalon (the posterior part of the forebrain) volume compared to PME girls. They also found similar group differences in the thalamus volume. When PME boys were compared with unexposed boys, increased volume in striatal and associated areas were found. When PME girls were compared with

unexposed girls, an increase in cortical thickness was observed. A considerable reduction in mid-posterior corpus callosum volume was found amongst PME girls when compared to unexposed girls. A reduction of volume in this area of the brain suggests weaker connectivity between brain areas. These findings suggests that there is sex-dependent brain changes caused by PME (Roos et al., 2014).

2.3.4. Motor development and visual motor integration in prenatal methamphetamine exposure

Several studies found that during the early years of life imperative foundations are developed for outcomes during childhood and adulthood (Feinstein & Bynner, 2004). Although early childhood is a critical period for brain development, middle childhood also provides social and educational experiences that are crucial for long term developmental outcomes (Feinstein & Bynner, 2004). Various studies on the effect of PME on cognitive functioning in children have found that PME does impact cognitive development in children. The following domains have been identified as problematic areas of cognitive functioning in PME children: attention, memory, motor function and visual motor integration (Cernerud, Eriksson, Jonsson, Steneroth, & Zetterstrom, 1996; Chang et al., 2009; Chang et al., 2004; LaGasse et al., 2011; Smith et al., 2011). For the purpose of this thesis, the focus will only be on motor function and visual motor integration.

2.3.4.1. Motor function

The use of MA has been associated with impairments in motor function. Motor function refers to several forms of movement, including automatic repetitive actions such as

walking, running, reflex actions, semi-voluntary actions such as sneezing, and voluntarily actions such as picking up something or throwing something (Bradshaw & Mattingly, 1995). Fine motor skills require the coordination of small muscle groups involved in small movements, such as writing or drawing. Gross motor skills refer to large movements, where coordination of large muscle groups is required, such as running or kicking a ball. Motor development is fundamental to a child's development. Proper motor development is a foundational skill for a child's school readiness (Pienaar, Barhorst, & Twisk, 2013). Despite evidence that MA causes a decrease in motor skills in abusers, very little is known about the impact that MA has on the motor function of PME children (Chang et al., 2009).

The sensory system provides us with the means of perceiving the world, whereas the motor system, in turn, provides us with the means of acting on the world. The control of sensory systems largely occurs within the posterior regions of the brain, while cortical control of movement occurs largely in the anterior regions in interaction with motor regions of the brain. Sensory-perceptual information is processed in primary processing areas and integrated by secondary and higher order cortices. Actions are determined by the information coming from sensory associated areas, such as the parietal lobes and subcortical structures, which includes the cerebellum and the basal ganglia (Zillmer et al., 2008).

A longitudinal study by Cernerud et al. (1996) followed 65 (36 girls; 29 boys) PME children in Sweden from birth up to the age of 14 years in an attempt to examine the long-term effects of PME on the development of a child. Between the ages of 14 and 15 years old, information about their growth and school achievements was collected. This data was compared to the means of unexposed children born in the same year of the exposed group to determine whether PME children performed worse overall. They found that PME children experienced difficulty in motor development and struggled with physical activities (Cernerud

et al., 1996). Despite the extensive data that was collected from this Swedish study one should consider a few absent key methodological aspects that limit the strengths of the study's outcomes. For example, the study did not include a control group, neither did it consider the presence of confounding drug exposure, such as tobacco and alcohol.

The Infant Development, Environment, and Lifestyle (IDEAL) study is the largest longitudinal study on the effect of PME on neurobehavioral outcomes. The IDEAL study (LaGasse et al. 2011), recruited participants, between the ages of 0-36 months, from the USA and New Zealand (NZ). The USA had a total of 379 participants (183 PME and 196 unexposed) and NZ had a total of 180 participants (85 PME and 95 unexposed) for the study. The NICU Neurobehaviour Scale (NNNS) was used to examine motor function in infants. All participants were measured within five days of birth. They found that PME infants experienced low tone, under arousal, poorer quality in movement and increased stress. Their findings suggest that PME does effect motor development (LaGasse et al., 2011).

The IDEAL study also examined the effect of PME on cognitive and motor development in children between the ages of 1-3 years. Smith et al. (2011) suggested that motor development during the infancy stage is associated with visual perceptual and spatial skills. Since visual perceptual processing may be negatively affected by PME, PME children might be at risk for experiencing difficulties when it comes to tasks that requires the coordination of movement (Smith et al., 2011). The authors found that PME children displayed poorer fine motor development compared to unexposed children at the age of one year old, with the poorest performance observed in those children who were exposed to heavy MA use prenatally. However, at the age of 3 they found that both high- and low-dose groups experienced similar levels of motor function that was not different compared to controls (Kiblawi et al., 2013). From these results the authors concluded that PME has

modest motor effects at the age of 1 year, which are mostly resolved by the time the child reaches the age of 3 (Smith et al., 2011). The results of this study prove inconsistent with the results of neuroimaging studies on the effect of PME on motor development. For instance, a study by Chang et al. (2009) found significant impaired motor development in PME children at the age of 4 years (Chang et al., 2009).

A study by Wouldes et al. (2014) also contradicts the findings of Smith et al. (2011) by finding similar results as Chang et al. (2009). The authors conducted their study on 210 participants (103 PME; 107 unexposed) from NZ. All children were assessed on the Bayley Scales of Infant Development, second Edition (BSID-II) at the ages of 1, 2 or 3 years to measure their cognitive and motor performance. Children were also assessed with the Peabody Development Motor Scale, second Edition (PDMS-2) at the ages of 1 and 3 to measure their gross and fine motor performance. They found that PME children experienced poorer fine and gross motor development when compared to unexposed children (Wouldes et al., 2014). It has been shown that children experiencing difficulty with motor coordination also experience difficulty with visual-motor coordination (Pienaar et al., 2013).

2.3.4.2. Visual-motor integration

Essential to various aspects of a child's development and cognitive development in middle childhood is the development of visual-motor integration. Visual-motor integration is the ability to integrate visual perceptual skills with fine motor coordination. Examples of visual-motor integration tasks include writing and drawing. Sensory-motor development is not only crucial for physical development, it is also essential for development in formal learning activities. It is suggested that deficits in visual-motor integration are precursors of learning disabilities and other neurological problems in later stages of life, for instance,

several important development qualities depend on the child's ability to perform visual-motor integration tasks (Lotz, Loxton, & Naidoo, 2005).

Studies performed on the effect of PME on the cognitive development showed that PME interferes with the development of visual-motor integration. As explained earlier, visual-motor integration is the integration between visual perceptual skills and fine motor skills, while studies have shown that PME children experienced impaired fine motor skills (Smith et al., 2011; Wouldes et al., 2014). A study by Chang et al. (2009) examined the brain metabolite levels and cognitive functioning in PME children between the ages of 3 and 4 years. Participants underwent proton magnetic resonance spectroscopy (1H-MRS) and were additionally evaluated with neuropsychological tests, such as the Beery VMI test. They found abnormal concentrations of brain metabolites in the thalamus and also found that PME children performed worse in visual-motor integration tasks. The authors suggested that a correlation exists between the abnormal brain metabolite concentrations they found in the frontal white matter and thalamus and poorer performance in visual-motor integration tasks (Chang et al., 2009). These findings were supported by Piper et al. (2011) who also found visual-motor integration impairment amongst PME children although when older, including 7-9-years-old. Thus it appears that a strong link exists between PME and impaired visual-motor integration.

Another study that provides evidence for the correlation between PME and impaired visual-motor integration development is that of Chang et al. (2004). The aim of their study was to assess the difference in structural brain volumes and cognitive function in PME children. The study included 28 participants (13 PME; 15 unexposed) between the ages of 3-16 years. The authors administered the Beery VMI test to assess visual motor integration, as well as the Purdue Pegboard Test to assess motor function in the children. The authors found

that PME children performed significantly poorer in the Beery VMI test, although they did not find any significant differences between groups when comparing the scores of the Purdue Pegboard test. The authors also found reduced volume in subcortical areas (putamen bilaterally, globus pallidus and caudate bilaterally) (Chang et al., 2004). These structures form part of the basal ganglia, a structure significantly involved in the generation of motor performance. Taking into account the reduced volume in subcortical structures, one can speculate that a link exists between the reduced volume in subcortical motor regions and impaired higher order visual-motor integration performance.

Table 2 summarises the findings, of the above studies on the effect of PME on motor development and visual motor integration. The table categorizes the findings according to the measurement that was used, the age of the children, and the outcome of the study.

Table 2: Motor development and visual motor integration in prenatal methamphetamine exposure

Author	Measurement	Age (years)	Outcome
Cernerud et al., 1996	Data from school results were collected from the following subjects: Swedish Language, Mathematics, and Physical Training	0-14	Poorer motor development and physical activities
Chang et al., 2004	Beery VMI Test, Purdue Pegboard Test, The Developmental Neuropsychological Assessment, Test of Variable Attention, The Wechsler Preschool and Primary Scale of Intelligence-Revised, Vocabulary and Block Design- Wechsler Intelligence Scale for Children-Third Edition, Children's Memory Scale-Dot Location subtest, Expressive One Word, Picture Vocabulary Test-Revised, Peabody Picture Vocabulary Test-Third Edition, Children's Depression Inventory, Controlled Oral Word Association Test-FAS ,	3-16	Abnormalities in visual integration, attention/vigilance impulsivity, verbal memory
Chang et al., 2009	Beery VMI Test	3-4	Poorer motor development
LaGasse et al., 2011	NICU Neurobehaviour Scale	0-5 days	Decline in quality of movement; increased physiological stress
Piper et al., 2011	Spatial Span, Conner's Continuous Performance Test II, Memory Island, Wechsler Abbreviated Scale of Intelligence, Dot location, Family Pictures,	7-9	Minor deficits in spatial function; delays in visual-motor integration; decline in attention
Smith et al., 2011	Peabody Developmental Motor Scale Bayley Scales of Infant Development	1-3	Decline in motor performance at 1 year; no difference in fine motor performances were observed at the age of 3years
Wouldes et al., 2014	Bayley Scales of Infant Development Second Edition or Peabody Development Motor Scale	1-3	Poorer motor development

2.4. Chapter summary

In this chapter I reviewed the relevant literature pertaining to prenatal MA exposure (PME) on the developing foetus, and the structural and cognitive abnormalities that may present as a consequence of PME. Firstly, I provided the literature search strategy that was used to locate relevant literature. Secondly, I discussed the effect of PME in animal models, followed by a discussion of the effect of PME in humans (physical -, neuro- and cognitive development).

CHAPTER 3

METHODS

In this chapter, I discuss the research methods and procedures used to obtain and analyse data for the study.

3.1. Introduction

To reiterate, the aim of the study was to contribute to the small, but growing, body of research that focuses on the brain development and motor performance of prenatally MA exposed children. The objective of the study was to determine whether PME children, between the ages of 8-9 years old, experienced differences in motor functioning when compared to unexposed children. The second objective was to determine whether PME children had different brain volumes and cortical thickness when compared to unexposed children. The thirds objective was to determine whether a correlation exists between brain volumes, cortical thickness and cognitive motor scores.

3.2. Research design

Data analysis was performed on data that was collected as part of a larger study. Primary data collection was coordinated by Dr Annerine Roos (supervisor). The candidate attended practical sessions on all aspects of data collection and analysis. The study had a cross-sectional quasi-experimental case-control design. Data were collected data at two time points: 1) when the participants were 6 years old and 2) again at the age of 8 years. The aim of the study was to assess the effect of PME on the developing brain over the period of two years. For the purpose of this study, only the data collected at the age of 8 was used. Two sets of quantitative data were used: set 1: structural brain data captured by means of a sMRI scan

and set 2: cognitive scores on motor performance captured by means of the Beery VMI test and Grooved Pegboard Test.

3.3. Setting

Data was collected from children attending a local school and day care centre in the northern suburbs of Cape Town. The day care centre is located in Leonsdale, Elsies River, where residents face high rates of crime (especially drug related crime) and unemployment. Between the years of 2015 and 2016, a total of 2 903 drug-related crimes were reported to the South African Police Service (SAPS) in this community alone (Crimestatssa, 2016).

It is important to bear in mind that PME might not be the only influential factor on motor and neurodevelopment in this specific population. The adverse effects of poverty on early child development (cognitive as well as neurodevelopment) are well known (Barnett, 1998; Bellows et al., 2017; Engle & Black, 2008). The majority of the children came from low-income households, with an average household income of R10 000 – R20 000 per year. A recent study in the USA, by Bellows et al. (2017), assessed the effect of poverty on motor development. They found that even though motor development is inherently established during early childhood, different environmental factors, such as poverty, also influence the process of motor development (Bellows et al., 2017). Even though a wide range of contextual factors might have an impact on the development of the child, for the purpose of this study only the impact of PME was considered.

3.4. Participants and procedure

Participants were children between the ages of 8-9 years. A total of 33 children were recruited for the study; 17 PME (age M = 8.52) and 16 unexposed children (age M = 8.27). The children were grouped according to their PME-status and matched according to their socio-economic background, age and gender. Parents/caregivers were presented with questionnaires in order to obtain demographic data on the participants.

The school and resident social worker assisted with identifying potential participants (PME and unexposed) for the study. Once the participants were identified, parents/caregivers were contacted by a research assistant (RA) to verify information and to invite the family to participate in the study.

Participants were excluded from the study if any of the following criterions were met: genetic anomalies, a history of neurological disorders, serious head injuries or premature birth (less than 36 weeks gestation). The research team also attempted to exclude children that were prenatally exposed to other substances from the study.

Once all participants were identified, a RA from the Cape Universities Brain Imaging Centre (CUBIC) contacted the parents/caregivers. Once the parents/caregiver agreed to participate in the study an appointment was schedule with each individual participant, as well as his/her caregiver.

When participants and their parent/caregiver arrived at CUBIC they were reintroduced to the study by the RA. The RA then explained to them the purpose and procedure of the study, as well as about their right to privacy and confidentiality of data. Ethical considerations are discussed in detail under section 3.7. Verbal assent was then obtained from the child and written consent from the parent/caregiver (See Appendix D and E). The parent/caregiver was asked to provide a detailed medical, socio-economic and

demographic history of the child. In some instances where the biological mother was unable to attend, collateral information was gathered via a family member/caregiver that was present. Where possible, a telephonic interview was held with the mother to confirm collateral information. The anthropometrics of the child was also captured, which included measurements of their weight, height and head circumference.

After completion of the interview with the parent/caregiver and participant, the child was prepared for the study. Cognitive assessment preceded scanning on the same day during the morning, in order to have optimised alertness. A psychology master's student (of the University of Cape Town) trained in the relevant tests collected and recorded the data from the Beery VMI test and the Grooved Pegboard tests at the Department of Psychiatry at Stellenbosch University.

Prior to the cognitive assessments, each participant was explained the procedure of the relevant assessment. Participants were provided with information regarding the content, duration and purpose of the assessment. Participants were also given the opportunity to ask questions prior to commencement of the assessment. Instructions to the assessments were given to participants in their home language, which was either Afrikaans or English. The assessor allowed for breaks during the session as required.

The scan session followed cognitive assessment after a break during which food was provided. A mock scanner that simulated the actual scanner was used. It was taken into consideration that the scanning situation may provoke anxiety, due to the loudness of the scanner during scan acquisition; therefore the RA was trained to familiarize the child with the scanning procedure. The success of the scan depended on minimal movement. As such, it was imperative that the scan process was simulated for the children to encourage minimal movement and minimize distress. The simulation process included a demonstration of

positioning as well as playing pre-recorded audios to familiarize the children with the sound of the scanner. During the scanning process, children were provided with the option of selecting an animated movie to watch.

After the assessments and brain imaging sessions were completed, both parent/caregiver and participant was debriefed and thanked for their participation, and received gifts as a token of appreciation for participation. They were then given the opportunity to ask any questions and/or express opinions regarding the study.

3.5. Measures

The measures that were used for the current study are discussed in the following section.

3.5.1. Background questionnaire

The purpose of the background questionnaire was to obtain demographic data about the child (socio-economic status (SES), anthropometric details, level of education), as well as the biological mother (SES, employment status, level of education, marital status). (See Appendix A)

3.5.2. Methamphetamine, alcohol and smoking exposure questionnaire

A questionnaire on MA use during pregnancy was administered to the biological mother/caregiver. The objective of the questionnaire was to gather information on the use of MA during pregnancy, the frequency of MA used during pregnancy, as well as the stage of pregnancy that MA was used. The questionnaire also queried the use of other substances during pregnancy such as alcohol and smoking. (See Appendix B)

3.5.3. Structural brain data

A Siemens Allegra 3T MRI scanner was used to acquire structural brain imaging of all participants. A high resolution structural scan (Van Der Kouwe, Benner, Salat, & Fischl, 2008) was attained that had the following parameters: repetition time of 2530ms; 4 echo times of 1.5ms, 3.2ms, 4.8ms and 6.5ms; flip angle of 7°; matrix size of 224x224x144; field of view of 224mm; voxel size of 1.3x1.0x1.0mm and acquisition time of 5 min 20 s. To track and correct subject motion in real time, the sequence used an echoplanar imaging volumetric navigator.

After structural imaging was completed, raw data from the scans was processed using Freesurfer 5.1.0. Freesurfer was applied on a supercomputing cluster at the Centre for High Performance Computing (CHPC, Cape Town). To determine volumes and cortical thickness, Freesurfer provides white matter and cerebral cortex templates to reconstruct raw data obtained from MRI scans. In order to acquire data on structural volumes, Markov random field theory is applied which segments brain areas into different tissue classes (Desikan et al., 2006). To perform thickness measurements, the cerebral cortex is divided into different areas, as defined by gyral and sulcal structures. Cortical thickness is calculated by measuring the closest distance between the white or gray matter boundary and the gray or cerebrospinal fluid boundary at each vertex on the image (Fischl & Dale, 2009).

3.5.4. Cognitive measures

3.5.4.1. The Beery developmental test of visual-motor integration (Beery VMI)

The Beery VMI test (4th edition) (Beery, 1997) is a test designed to examine the extent to which children, aged 2 years and older, are capable of integrating their visual and

motor abilities. Participants are expected to copy 27 geometrical designs as precisely as possible. The difficulty level of the designs ranges from very simple to fairly complex. The Beery VMI also includes two supplemental tests that are used to assess aspects of motor coordination and visual perception. The same 27 geometric forms from the main test are used in the two supplementary tests. The Motor Coordination Test requires of the participant to copy the stimulus forms, using a pencil, without crossing the double-lined paths. The Visual Perception Test requires of the participant to identify the exact match, for each of the 27 geometric forms, from a variety of similarly-shaped forms (Beery, 1997). The cross-cultural validity of the Beery VMI test has not yet been the determined, although Brown and Rodger (2008) argue that this test is culture-free since it makes use of shapes rather than numbers and letters. It has been determined that sex, socio-economic status, ethnicity and place residence does not affect the outcome of performance (Brown & Rodger, 2008).

3.5.4.2. Grooved Pegboard Test (GPT)

The Grooved Pegboard Test (GPT) is a test used to assess complex visual-motor coordination. The test consists of a pegboard, containing 25 holes, with randomly positioned slots. Pegs, with a key on one side must then be rotated to match the slot at a hole before the peg can be inserted. Participants are then expected to place all pegs into the 25 holes. They have to pick up one peg at a time and place them into the hole. Participants can use only one hand at a time, starting with their dominant hand and then switching over to their non-dominant hand. The results of the test are determined by measuring the time it took the participant to complete the first line of the board, the entire board, the number of times the pegs were dropped, and the number of pegs placed. The validity and reliability of the GPT has been well-established (Ruff & Parker, 1993). It has been verified that the GPT is valid for

use with South African populations. Race, language and sex do not greatly affect the outcome of the test (Ferett et al., 2014).

3.6. Data analysis

SPSS, version 22.0., was used to analyse all of the data for this study. The analysis entailed multiple steps. Firstly, the unexposed group and the PME group were compared based on socio-demographic variables, anthropometric variables, as well as maternal sample characteristics. Levene's test was used for all continuous variables to assess homogeneity of variance across the two groups. The homogeneity of variance was assumed if the *p* value was greater than 0.05. The Kolmogorov-Smirnov test was implemented to test whether the data distributions were normal. When the data distributions were normal, either the Pearson Chi-square test or the independent sample *t*-test, depending on the type of data, was used to test for differences between the PME and the unexposed group. In cases where data were non-parametric, the Mann-Whitney *U*-test was implemented.

In the next part of the analysis, the first objective of the study was investigated: to determine whether PME children would perform worse in cognitive motor tests compared to the unexposed group. For both cognitive tests the scoring procedures outlined in the test administration manuals were used (Beery, 1997; Trites, 1977). Scores for the Beery VMI test were standardized, whereas the insert and removal times for the GPT were used. Analysis began with exploring the data and testing the assumptions that underlie inferential analysis. The Shapiro-Wilk and the Levene's test were used to assess whether the assumptions of normality and homogeneity were upheld for all cognitive outcomes. In cases where data was not normally distributed, the non-parametric Mann-Whitney *U*-test was used; otherwise the independent sample *t*-test was used.

To ensure that the observation of any cognitive deficits, amongst the PME group, were as a result of PME, and not the effect of potential confounding variables, hierarchical regression analysis was applied to the data.

A correlation matrix was constructed in order to start the investigation of the relationship between PME, cognitive outcomes, and potential confounding variables. The aim of the correlation matrix was to examine associations between all the different cognitive outcomes and potential confounding variables. All socio-demographic variables that differed significantly between groups were selected as potential confounding variables. Based on the results of tests of normality, by means of the Levene Test, either the Pearson *r* coefficient test or the Spearman ρ coefficient test was used. After the construction of the correlation matrix, potential confounding variables that had a significant correlation with the cognitive outcomes was identified. To investigate the degree to which the association between PME and cognitive outcomes are influenced by the confounding variables, a separate hierarchical regression analysis was conducted for each one of the five cognitive outcomes.

In the next part of the analysis, the second objective was investigated: to determine whether PME children would show a difference in brain volumes and cortical thicknesses of regions involved in motor function compared to unexposed children. The structural brain data that was used is data that has been processed using Freesurfer 5.1.0. All data is presented in cubic centimetres (cm^3). The first step of the analysis was to test for homogeneity and normality of the data. Once that was determined, either ANOVA or the Mann-Whitney *U*-test was used to test for significant differences between groups. Since the main aim of this study is to determine differences in motor function in PME children and unexposed children, there was a specific focus on those areas of the brain responsible for motor function as derived from the literature. Therefore, the Regions of Interest (ROI) was the motor centres and

associated areas. Both the brain volumes and cortical thickness of these areas were taken into consideration.

In the next step, a correlation matrix was constructed to ensure that any difference in brain structures, which was observed amongst the PME group, is the result of PME, and not the effect of potential confounding variables. The aim of the correlation matrix was to examine associations between all the significant brain structures and potential confounding variables. Socio-demographic variables that indicated a significant difference between groups and that also might have had a potential impact on brain structures, were selected as potential confounding variables. Based on the results of tests of normality, by means of the Levene's Test, either the Pearson *r* coefficient test or the Spearman ρ coefficient test was used. In the case where the correlation matrix shows that a significant correlation exists between a certain brain structure and a confounding variable, a separate hierarchical regression analysis was conducted for that variable.

Lastly, objective 3 that aimed to determine whether the impairment in cognitive motor scores, which was observed amongst the PME group, is caused by alteration in brain volume/cortical thickness was examined. A correlation test between motor scores and brain data was performed. Only the data from the PME group were included in this test. Where data was parametric, the Pearson *r* coefficient test was used. In the case where the data was non-parametric, the Spearman ρ test was used.

3.7. Ethical consideration

This study was approved by the Human Research Ethics committees of the University of Cape Town and Stellenbosch (ethics number: HREC 235/2009 / UCT 7 and SU-HSD-

002904) (See appendix C). The study was conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, the South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research. In keeping with ethical requirements, parental or caregiver consent as well as assent from the participants was obtained prior to data collection.

3.7.1. Confidentiality

Parents/caregivers were assured that only members of the research team would have access to the collected data. Permission was granted to me, by the Ethics Committee to access the data – delinked from identifying characteristics such as names. Furthermore, all parents/caregivers were assured that the data would remain confidential and in the case of publication, none of the participants would be identified at any time. All personal details of the participants, in the study, were coded to keep information confidential and anonymous.

3.7.2. Anonymity

Consideration was given to the protection of the identity of the aftercare facility and the participants of the study. Data that was given to me, by the primary supervisor, was delinked from identifying features such as names, surnames and addresses. Names of children were replaced by a unique participant number. Complete anonymity was assured at all times.

3.8. Chapter summary

The chapter started with a brief introduction of the aim of the current study. This was followed by a discussion of the measures that were used to obtain data. These measures included a demographical questionnaire, a questionnaire on the use of MA during pregnancy, MRI scans to obtain structural brain data and cognitive motor assessments, which included

the Beery VMI test and the Grooved Pegboard Test. Subsequently, the procedures that were followed to obtain data were discussed. A discussion on the data analysis that was used was also given. Lastly, a discussion was presented on the relevant ethical considerations. The following chapter will present the results that were obtained from the analysis of the study.

CHAPTER 4

RESULTS

I start this chapter with the presentation of the child sample characteristics, which includes socio-demographic variables, as well as the anthropometric variables. This is followed by a presentation of the maternal sample, which includes socio-demographic variables and details on substance use during pregnancy. In the next section of the chapter I present the results pertaining to the statistical analysis of the cognitive outcomes and structural brain data between the PME and the unexposed group.

4.1. Child sample characteristics

A total of 33 children took part in the study; 17 PME and 16 unexposed. The groups were matched according to their socio-economic background, gender and age. (See Table 3)

4.1.1. Socio-demographic variables of sample

Table 3 presents the socio-demographic information of PME and unexposed children and Table 4 the maternal sample characteristics. The assumption of normality and homogeneity were upheld for all the data distributions, except where otherwise stated.

4.1.1.1. Age

The ages of the children ranged between 8-9 years, with a mean (M) of 8.14 years across groups. The mean age for the PME group was 8.02 years and 8.27 years for the unexposed group. Although there was no significant between group difference in age; participants in the unexposed group were slightly older than those in the PME group.

4.1.1.2. Gender

A total of 17 participants (51.52%) were boys and 16 participants (48.48%) were girls. The PME group consisted out of 10 males (58.82%) and 7 females (41.18%). The unexposed group consisted out of 7 males (43.75%) and 9 females (56.25%). The Pearson Chi-squared test detected no significant between group differences regarding gender.

4.1.1.3. Language

The home language of the children was either Afrikaans (N = 25; 75.75%) or English (N = 8; 24.25%). From the PME group, Afrikaans was the most prevailing language (N = 13; 76.47%) and English less so (N = 4; 23.53). Amongst the unexposed group Afrikaans (N = 12; 75%) was also the most prevailing language and English less so (N = 4; 25%). Even though the majority of the participants were Afrikaans, the Pearson chi-squared test detected no significant between group differences regarding language.

4.1.1.4. Level of education

This variable was defined as the grade in which the participant was enrolled at the date of testing. From the total sample group, children were either in grade 1 (N = 1; 3.03%), grade 2 (N = 14; 42.45%), grade 3 (N = 17; 51.5%) or grade 4 (N = 1; 3.03%). The PME group was enrolled in grades as follows: grade 2 (N = 10; 58.82%), and grade 3 (N = 7; 41.18%). The unexposed group was enrolled in grades as follows: grade 1 (N = 1; 6.25%), grade 2 (N = 4; 25%), grade 3 (N = 10; 62.5%) and grade 4 (N = 1; 6.25%). The Pearson's chi-squared test did not detect any significant difference between the two groups according to the level of education.

4.1.2. Anthropometric variables

Depending on the distribution of the data, the independent sample t-test or the Mann-Whitney test was used to test for any significant differences between groups regarding weight, height, and head circumference.

4.1.2.1. Handedness

All participants, from both groups, were right-handed, therefore no tests were performed to detect significant differences between groups.

4.1.2.2. Weight

The assumption of normality was not upheld for the data distribution on weight. The weight of both the unexposed children, $D(16) = 0.27$, $p < 0.01$, and exposed children, $D(17) = 0.25$, $p < 0.01$, were significantly non-normal, therefore the Mann-Whitney test was implemented. No significant difference in weight was detected between groups.

4.1.2.3. Height

The assumption of the homogeneity of variance was not upheld, $F(1, 31) = 5.61$, $p < 0.05$, therefore the Mann-Whitney test was implemented. A significant difference was detected in height between the two groups ($p = .031$).

4.1.2.4. Head circumference

The assumption of normality and homogeneity of variance was upheld; therefore the independent sample *t*-test was used. No significant difference was detected between groups regarding head circumference.

Table 3

Socio-demographic and anthropometric principles of child sample

Variable	Group PME (N = 17)	Group Unexposed (N=16)	t / X^2	p	ESE
Socio-demographic Variables					
Age	8.02 (0.47)	8.27 (0.36)	1.68	.10	0.59
Gender (M : F)	10:7	7:9	0.75	.49	0.15
Language (Afr : Eng)	13:4	12:4	0.01	.92	0.17
Education (Gr1:Gr2:Gr3:Gr4)	0:10:7:0	1:4:10:1	5.08	.17	0.39
Anthropometric Variables					
Handedness (L : R)	0:17	0:16			
Weight (kg)	22.46 (2.98)	25:37 (7.09)	1.56	.13	0.32
Height (cm)	122.82 (4.13)	127.36 (7.54)	2.16	.04*	0.33
Head circumference[A]	51.43 (1.36)	52.25 (1.87)	1.42	.17	0.50

Note: Some cells contain mean values with standard deviations in brackets; others contain data presented in ratios. PME = prenatal methamphetamine exposure; ESE = estimate of effect size; M = male; F = female; Eng = English; Afr = Afrikaans; Gr = grade; L = Left; R = Right. r or ϕ Was used to calculate the estimate of effect size, depending on whether the Chi-squared test or independent sample *t*-test was used.
[A] Data for 1 unexposed child was missing
*$p < 0.05$.

4.2. Maternal sample characteristics

Either an independent sample *t*-test or a Pearson Chi-squared test was used, as appropriate, to determine whether a significant difference exists between group differences regarding socio-demographic characteristics of the mother, as well as whether or not alcohol and cigarettes were used during pregnancy (see Table 4).

4.2.1. Education

An independent sample t-test was used to test for significant group differences regarding level of education. Even though the average education level was higher in the

unexposed group (M = 10 years) compared to the PME group (M = 9 years), no significant difference was detected.

4.2.2. Employment

A Pearson Chi-Squared test detected a significant between group difference regarding employment status of the mothers ($p = 0.029$), a difference associated with a moderate effect size. A minority of mothers from the PME group was employed (18.75%), while significantly more mothers of the unexposed group were employed (60%).

4.2.3. Primary caregiver

Amongst the 17 PME children, 4 (23.53%) children had their mother as their primary caregiver, 10 (58.82%) had their grandmother, 2 (11.76%) had both their mother and grandmother and 1 (5.88%) had their aunt as their primary caregiver. Amongst the 16 unexposed group, 14 (87.5%) children had their mother as their primary caregiver, 1 (6.25%) had their aunt and 1 (6.25%) had both their aunt and grandmother as primary caregivers. A significant group difference was detected, by means of the Pearson Chi-squared test, with regards to who the primary caregiver was ($p = 0.001$), a difference associated with a large effect size. Thus, the majority of the PME children had their grandmother as a primary caregiver (58.82%), whereas the unexposed children were more likely to have their mother as the primary caregiver (87.5%).

4.2.4. Marital status

The Pearson Chi-squared test detected significant between group differences regarding marital status ($p = 0.023$), a difference associated with a large effect size. In the PME group, none of the mothers were married, while the majority of them were single (80%).

Less mothers in the unexposed group were single (40%), while almost half of the mothers were married (46.67%).

4.3. Substance use amongst maternal sample

It is important to note that the data on alcohol and cigarette use during pregnancy was categorical in nature. For this reason, the Pearson Chi-square test was implemented to test for significance. The questionnaire that was used to test for substance use during pregnancy did query on the duration, frequency and amount of MA, alcohol and cigarette use during pregnancy; however due to the difficulty in collecting accurate retrospective information, most of the participants only answered ‘yes’ or ‘no’ to questions regarding prenatal substance use.

4.3.1. Alcohol use

No significant difference was detected between groups on alcohol use during pregnancy. Only a small number of mothers consumed alcohol during pregnancy, from both the PME group (4; 23.5%) and the unexposed group (1; 6.25%). However, mothers in the unexposed group abstained from using alcohol after trimester one of pregnancy.

4.3.2. Cigarette use

A significant difference was detected between groups on cigarette use during pregnancy (p = .02). The majority of mothers in the PME group smoked during pregnancy (76.47%), while considerably fewer mothers in the unexposed group smoked during pregnancy (31.25%).

Table 4

Maternal sample characteristics

Variable	Group		t / X^2	P	ESE
	PME (N=17)	Unexposed (N=16)			
Education (years)[A]	9 (1.27)	10 (2.17)	1.58	0.125	0.56
Employment (yes/no) [A]	3:13	9:6	5.55	0.029*	0.42
Primary caregiver	4:10:2:1:0	14:0:0:1:1	18.85	0.001***	0.75
Mother (n, %)	4 (23.53)	14 (87.5)			
Grandmother (n, %)	10 (58.82)	0 (0)			
Mother/Grandmother (n, %)	2 (11.76)	0 (0)			
Aunt (n, %)	1 (5.88)	1 (6.25)			
Aunt/Grandmother (n, %)	0 (0)	1 (6.25)			
Marital status[A]	12:0:1:1:1	6:7:2:0:0	11.33	0.023*	0.62
Single (n, %)	12 (80.00)	6 (40.00)			
Married (n, %)	0 (0)	7 (46.67)			
Living with partner (n, %)	1 (6.67)	2 (13.33)			
Divorced (n, %)	1 (6.67)	0 (0)			
Widowed (n, %)	1 (6.67)	0 (0)			
Substance Use					
Alcohol Use (yes/no)	4:13	1:15	1.91	.34	0.058
Cigarette Use (yes/no)	13:4	5:11	6.80	.02*	0.394

Note: Some cells contain mean values with standard deviations in brackets; others contain data presented in ratios. PME = prenatal methamphetamine exposure; ESE = estimate of effect size; r or ϕ Was used to calculate the estimate of effect size, depending on whether the Chi-squared test or independent sample t-test was used.
[A]Data missing for one mother in the PME group, and one mother in the unexposed group.
*$p < 0.05$. ***$p < 0.01$.

4.4. Cognitive outcomes

Objective 1: To determine if PME children are impaired on measures of visual-motor integration and coordination, when compared to unexposed children. In order to determine

this data from the Beery VMI test and the GPT were explored and assessed for between group differences using descriptive statistics.

Q-Q plots showed potential departures from normality for a few of the cognitive outcome variables. The Shapiro-Wilk and the Levene's test were then used to assess whether the assumption of normality and homogeneity was upheld for all cognitive outcomes (See Table 5).

Table 5

Results for tests of normality and homogeneity of variance for cognitive variables (N=33)

Cognitive Measure	Shapiro-Wilk Test		Levene's Test
	PME	Unexposed	
Beery VMI (Total Score)	.14	.64	.63
Visual Score	.35	.03*	.68
Motor Score	.22	.95	.42
Grooved Pegboard Test			
Insertion time DH	.40	.28	.21
Insertion time NDH	.33	.16	.02*

Note. Data presented are *p*-values. PME = prenatal methamphetamine exposed; VMI = visual motor integration; DH = dominant hand; NDH = non-dominant hand. *$p < 0.05$.

The assumption of homogeneity, as assessed by the Levene's statistic was upheld for all variables, except for the Beery VMI test visual score. The GPT non-dominant hand insertion time violated the assumption of normality, as assessed by the Shapiro-Wilk Test.

Table 6 presents descriptive statistics as well as results of between group comparisons for the cognitive outcome variables. Analysis of the data showed that children in the PME group performed poorer, in both cognitive tests, compared to children in the unexposed group. In the Beery VMI visual test, no significant difference was detected between PME children (M = 5.47) and unexposed children (M = 6.75). A significant difference ($p < .03$) was

detected between groups in the motor scores. Although overall, children in the PME group scored lower in the Beery VMI test, this was not significantly different compared to the unexposed group. Children in the PME group performed worse, as expected, in the GPT. A significant difference ($p < .04$) was detected between groups in the 'insertion time with the non-dominant hand'. There was a trend for poorer performance in the PME group in 'insertion time with dominant hand' ($p = .07$), compared to the unexposed group.

Table 6

Cognitive outcome variables: descriptive statistics and between group assessments (N = 33)

	Group					
Cognitive Measure	PME (n = 17)	Unexposed (n = 16)	*t / U*	*df*	*P*	ESE
Beery VMI (Total Score)	8.24 (3.11)	8.81 (2.74)	.56	31	.57	0.19
Visual Score [A]	5.47 (2.53)	6.75 (3.13)	97.00	31	.16	0.45
Motor Score	8.18 (1.74)	9.75 (2.24)	2.26	31	.03*	0.80
Grooved Pegboard Test						
Insertion Time DH	47.45 (12.90)	40.00 (9.90)	1.85	31	.07	0.65
Insertion Time NDH [B D]	59.06 (18.63)	46.83 (11.07)	2.20	29	.04*	0.80

Note. Data presented are p-values. PME = prenatal methamphetamine exposed; VMI = visual motor integration; DH = dominant hand; NDH = non-dominant hand.
[A]Assumption of normality was not upheld, Mann-Whitney *U* test was used.
[B] Assumption of homogeneity was not upheld, Mann-Whitney *U* test was used.
[D] Data unavailable for 1 PME child and 1 unexposed child.
*p < 0.05.

4.5. Hierarchical regression analysis of cognitive outcomes

To ensure that the cognitive deficits that was observed amongst the PME group was the result of PME and not the effect of potential confounding variables, hierarchical regression analysis was applied to the data.

A correlation matrix was constructed in order to investigate the relationship between PME, cognitive outcomes, and potential confounding variables (see Table 7). Based on the results of Levene's tests of normality, variables that upheld the assumption of normality was that of the weight of the child, gender of child and maternal cigarette use during pregnancy. Variables that violated the assumption of normality were that of height of child, maternal education level, and maternal employment status.

As indicated by Table 7, several of the potential confounding variables correlated significantly with the cognitive outcomes. Based on literature, the correlation matrix, and significant between group differences six predictors were selected for inclusion in the regression model: height of the child, weight of the child, gender, employment status of the mother, maternal cigarette use during pregnancy, and PME-status. While no significant between group difference were detected regarding weight (see Table 3), previous studies have found that PME children weigh significantly less compared to their unexposed peers (Smith et al., 2015). Therefore, weight was included in this regression model. Even though no significant difference was detected between groups regarding gender, it was included in the regression model since various studies have confirmed that gender influences cognitive functioning (Weiss, Kemmler, Deisenhammer, Fleischhacker, & Delazer, 2003). Alcohol use during pregnancy was not included in the regression model since all mothers that used alcohol, also smoked.

Table 7

Correlation between cognitive outcomes and potential confounding variables (N=33)

	Child			Mother	
Cognitive Measure	Height[A]	Weight[B]	Gender [B]	Employment[A]	Cigarette Use[B]
Beery VMI (Total Score)	-.07	.18	-.32	.07	-.26
Visual Score	.02	.05	-.10	.29	-.12
Motor Score	.14	.17	.05	.46**	-.35*
Grooved Pegboard Test					
Insertion Time DH	-.01	-.07	.29	-.17	.27
Insertion Time NDH	-.13	-.28	.44*	-.25	.38*

All tests are 2-tailed. VMI = visual-motor integration; DH = dominant hand; NDH = non-dominant hand.
[A]Statistics presented is Spearman correlation coefficients (ρ)
[B]Statistic presented is Pearson correlation coefficients (r)
$\Delta\ p < .10$. *$p < .05$. **$p < .01$.

To investigate the degree to which the association between PME and cognitive outcomes were influenced by the above mentioned confounding variables, a separate hierarchical regression analysis was conducted for each one of the five cognitive outcome variables. The confounding variables that were controlled for (child height, child weight, child gender, maternal employment status, maternal cigarette use) were entered at the first step of the model as a block. In the second step, the exposure status was included.

Model 1: Predicting performance on the Beery VMI score:

Table 8 shows that none of the potential confounding variables contributed significantly to the Beery VMI test scores, $F(5, 25) = 2.36$, $p = .09$. In the final model, upon adding PME-status, none of the predictors were significant, $F(6, 24) = 2.04$, $p = .10$.

Table 8

Hierarchical regression model 1: performance on the Beery visual-motor integration test, predicted by confounding variables and PME status (N=33)

Variable Entered	*B*	*SE B*	*B*
Step 1			
Constant	27.19	12.17	
Height	-.137	.109	-.302
Weight	.000	.000	.249
Gender	-2.34	.984	-.407
Employment	.734	1.05	.492
Cigarette use	-2.484	1.055	-.427
Step 2			
Constant	25.741	12.409	
Height	-.129	.110	-.285
Weight	.000	.000	.254
Gender	-2.315	.992	-.403
Employment	.1.003	1.115	.170
Cigarette use	-2.815	1.146	-.484
PME Status	.915	1.179	.159

Index: $R^2 = .19$ for Step 1, $\Delta R^2 = .17$ for Step 2. ($p = .10$)

Model 2: Predicting performance on the Beery VMI (Visual) test score:

Table 9 shows that the set of potential confounding variables was not a significant predictor of the Beery VMI (Visual) test scores, $F(5,25) = 1.06$, $p = .40$. The introduction of PME-status, in the second step, did not significantly alter the outcome, $F(6,24) = .90$, $p = .51$.

Table 9

Hierarchical regression model 2: performance on the Beery visual-motor integration test (visual scores), predicted by confounding variables and PME status (N=33)

Variable Entered	*B*	*SE B*	*B*
Step 1			
Constant	9.311	13.53	
Height	-.008	.121	-.018
Weight	-2.30	.000	-.044
Gender	-1.35	1.09	-.235
Employment	2.29	1.17	.384
Cigarette use	-.655	1.173	-.112
Step 2			
Constant	10.35	13.90	
Height	-.014	.123	-.030
Weight	-2.50	.000	-.048
Gender	-1.37	1.11	-.237
Employment	2.09	1.25	.352
Cigarette use	-.417	1.28	-.071
PME Status	-.657	1.32	-.113

Index: $R^2 = .16$ for Step 1, $\Delta R^2 = .18$ for Step 2. ($p = .51$)

Model 3: Predicting performance on the Beery VMI (Motor) test score:

Table 10 shows that none of the confounding variables contributed significantly to the Beery VMI test (motor scores), $F(5, 25) = 1.53$, $p = .22$. In the final model, upon adding the PME-status, none of the predictors were significant, $F(6, 24) = 1.31$, $p = .29$.

Table 10

Hierarchical regression model 3: performance on the Beery visual-motor integration test (motor scores), predicted by confounding variables and PME status (N=33)

Variable Entered	*B*	*SE B*	*B*
Step 1			
Constant	11.17	9.63	
Height	-.018	.086	-.052
Weight	2.31	.000	.060
Gender	-.282	.778	-.066
Employment	1.48	.832	.338
Cigarette use	-1.24	.835	-.287
Step 2			
Constant	12.12	9.86	
Height	-.023	.088	-.067
Weight	2.13	.000	.055
Gender	-.298	.788	-.070
Employment	1.31	.886	.298
Cigarette use	-1.03	.911	-.237
PME Status	-.599	.936	-.140

Index: $R^2 = .23$ for Step 1, $\Delta R^2 = .25$ for Step 2 ($p = 29$)

Model 4: Predicting performance on the Grooved Pegboard Test (Dominant Hand Scores):

Table 11 shows that none of the confounding variables contributed significantly to the GPT (Dominant Hand) scores, $F(5,25) = 1.42$, $p = .25$. In the final model, upon adding die PME-status, none of the predictors were significant, $F(6,24) = 1.77$, $p = .15$.

Table 11

Hierarchical regression model 4: performance on the Grooved pegboard test (dominant hand scores), predicted by confounding variables and PME status (N=33)

Variable Entered	*B*	*SE B*	*β*
Step 1			
Constant	-49.63	54.61	
Height	.733	.489	.336
Weight	-.001	.001	-.238
Gender	8.21	4.41	.341
Employment	-4.50	4.72	-.182
Cigarette use	7.06	4.74	.289
Step 2			
Constant	-63.32	53.206	
Height	.805	.473	.423
Weight	.000	.001	-.226
Gender	8.44	4.25	.350
Employment	-1.96	4.78	-.079
Cigarette use	3.92	4.92	.161
PME Status	8.67	5.05	.360

Index: $R^2 = .22$ for Step 1, $\Delta R^2 = .30$ for Step 2 ($p = .15$)

Model 5: Predicting performance on the Grooved Pegboard Test (Non-Dominant Hand Scores):

Table 12 shows that none of the confounding variables contributed significantly to the GPT (Non-Dominant Hand) scores, $F(5, 23) = 4.19$, $p = .11$. In this model, gender ($p = .004$) and maternal cigarette use during pregnancy was a significant predictor of this cognitive outcome ($p = .02$). In the second step, where PME-status is introduced to the model, none of the predictors were significant, $F(6, 22) = 3.99$, $p = .15$. In the second step of the model, gender ($p = .003$) was a significant predictor of this cognitive outcome.

Table 12

Hierarchical regression model 5: performance on the Grooved pegboard test (non-dominant hand scores), predicted by confounding variables and PME status (N=33)

Variable Entered	*B*	*SE B*	*β*
Step 1			
Constant	-62.71	62.13	
Height	.909	.556	.358
Weight	-.001	.001	-.383
Gender	16.2	5.10	.492**
Employment	-7.30	5.34	-.219
Cigarette use	13.58	5.39	.412*
Step 2			
Constant	-73.28	61.20	
Height	.941	.545	.370
Weight	-.001	.001	-.359
Gender	16.9	5.01	.512**
Employment	-4.41	5.59	-1.33
Cigarette use	9.98	5.84	.303
PME Status	8.96	6.25	.27

Index: $R^2 = .48$ for Step 1, $\Delta R^2 = .52$ for Step 2 ($p = .15$)
*$p < .05$, ** $p < .01$

4.6. Brain volume and cortical thickness analysis

Objective 2: To determine if a difference exists in structural brain volumes and cortical thickness in the motor centres and associated areas of the brain when comparing structural brain data of MA exposed (8-9 years old) children to unexposed children.

The ROI was the motor centres and associated areas of the brain. Either ANOVA or the Mann-Whitney U-test was applied to those variables. Only variables that showed a significant difference between groups are reported (see Table 13). Other brain regions, which did not show any significant difference, were reported separately (See Appendix F). No significant difference in brain volume was detected between groups, however there was significant differences in ten cortical thickness variables (see Table 13).

Table 13

Group differences in cortical thickness (N = 33)

Cortical Thickness	Group PME (*n* = 17)	Group Unexposed (*n* = 16)	*t / U*	*df*	*P*
Frontal Structures					
LH Superior Frontal[A]	3.21 (.20)	3.36 (.13)	78.5	31	.04*
RH Superior Frontal	3.17 (.19)	3.31 (.18)	2.16	31	.04*
RH Caudal Middle-Frontal	2.79 (.30)	2.99 (.19)	2.22	31	.03*
RH Rostral Middle-Frontal	2.80 (.16)	2.92 (.15)	2.23	31	.03*
Temporal Structures					
LH Middle Temporal	3.07 (.19)	3.21 (.17)	2.25	31	.03*
LH Superior Temporal[A]	2.97 (.25)	3.11 (.18)	83	31	.05*
LH Parahippocampal	2.46 (.28)	2.86 (.25)	4.32	31	< .001***
RH Parahippocampal	2.58 (.31)	2.76 (.16)	2.07	31	.05*
Parietal Structures					
LH Superior Parietal	2.58 (.12)	2.67 (13)	2.13	31	.04*
Occipital Structures					
LH Cuneus	2.28 (.20)	2.43 (.19)	2.21	31	.04*

Note. Data presented are p-values. PME = prenatal methamphetamine exposed; LH = Left Hemisphere; RH = Right Hemisphere

[A]Assumption of normality was not upheld, Mann-Whitney *U* test was used.

*p < 0.05. ***p < 0.01

4.7. Correlation analysis of structural brain difference outcomes

To determine whether the difference in brain structures that was observed amongst the PME group is the result of PME, and not the effect of potential confounding variables, a

correlation test was applied to the data, that would be followed by a hierarchical regression analysis would any correlation be found to be significant.

A correlation matrix was constructed in order to start the investigation of the relationship between PME, brain outcomes, and potential confounding variables (see Table 14). Socio-demographic variables that indicated a significant difference between groups, and also might have had a potential impact on brain structures, were selected as potential confounding variables (see Table 3 and 4). Gender was also included as a potential confounding variable since changes within the frontal, striatal, and parietal areas are to some extend gender dependent (Gomes-da-silva et al., 2004). The potential confounding variables that was identified was: height of child, gender of child and maternal cigarette use during pregnancy.

Table 14

Correlation between cortical thickness and potential confounding variables (N=33)

Cortical Thickness	Height of Child[A]	Gender of Child[B]	Maternal Cigarette Use[B]
Frontal Structures			
LH Superior Frontal	.05	.23	.001
RH Superior Frontal	-.15	.30	.04
RH Caudal Middle-Frontal	-.05	.10	.06
RH Rostral Middle-Frontal	-.04	.15	-.05
Temporal Structures			
LH Middle Temporal	-.02	.05	.05
LH Superior Temporal	-.12	-.09	.19
LH Parahippocampal	.20	.14	-.28
RH Parahippocampal	.04	.22	.17
Parietal Structures			
LH Superior Parietal	-.03	.08	-.19
Occipital Structures			
LH Cuneus	-.07	.09	-.24

All tests are 2-tailed. VMI = visual-motor integration; DH = dominant hand; NDH = non-dominant hand.
[A]Statistics presented is Spearman correlation coefficients (ρ)
[B]Statistic presented is Pearson correlation coefficients (r)

As indicated by Table 14, none of the potential confounding variables had a significant correlation with the structural brain measures. Therefore, no hierarchical regression analysis was applied to test for the effect of potential confounding variables. From the results of the correlation matrix it was concluded that the socio-demographic variables had no influence on the outcome of the structural brain data.

4.8. Correlation between cognitive outcomes and cortical thickness

Objective 3: To determine if a correlation exists between the structural brain data and the results of the Beery VMI test and the GPT in PME children.

The aim of this correlation test was to determine whether the alteration in cortical thicknesses, which was observed amongst PME children, was associated with poorer in performance in the Beery VMI test and the GPT. The focus of this test was on the PME group.

Table 15

Correlation between cognitive outcomes and cortical thickness in PME children (N=17)

	Cognitive Measure				
Brain Area	Beery VMI[A]	Beery Visual[B]	Beery Motor[A]	GPT Insert Time DH[A]	GPT Insertion Time NDH[B]
Frontal Structures					
LH Superior Frontal	.08	.14	.05	.15	-.04
RH Superior Frontal	-.04	-.06	-.13	.07	.03
RH Caudal Middle-Frontal	.04	.17	-.003	.09	-.03
RH Rostral Middle-Frontal	.02	.12	.05	.22	.05
Temporal Structures					
LH Middle Temporal	-.14	-.10	-.14	.35*	.32*
LH Superior Temporal	-.04	.16	-.07	-.02	.13
LH Parahippocampal	-.40*	-.20	-.19	.05	.12
RH Parahippocampal	-.36*	-.16	-.22	.09	.23
Parietal Structures					
LH Superior Parietal	.25	.31*	.13	-.21	-.13
Occipital Structures					
LH Cuneus	.47*	.06	-.03	-.24	-.20

All tests are 2-tailed. VMI = visual-motor integration; DH = dominant hand; NDH = non-dominant hand.
[A] Statistic presented is Pearson correlation coefficients (r)
[B] Statistics presented is Spearman correlation coefficients (ρ)
*$p < .05$.

A number of correlations were found between cortical thicknesses and cognitive outcomes. Left middle temporal thickness, correlated with GPT scores using the Dominant Hand ($r = .35$, $p < .05$). Thickness in this area also correlated with GPT scores using the non-Dominant Hand ($\rho = .32$, $p < .05$).

A correlation between the parahippocampal thickness and the Beery VMI test was detected. Beery VMI test total scores correlated with both left parahippocampal (r = -.40, p < .05), as well as right parahippocampal thickness (r = -.36, p < .05).

Left superior parietal thickness correlated with Beery Visual test scores (ρ = .31, p < .05). In turn, left cuneus thickness correlated with Beery VMI test total scores (r = .47, p = .05).

4.9. Chapter summary

The chapter started with the presentation of the child sample characteristics, which includes socio-demographic variables, as well as the anthropometric variables. This was followed by a presentation of the maternal sample, which include socio-demographic variables and details on substance use during pregnancy. In the next section of the chapter I presented the results pertaining to the statistical analysis of the cognitive outcomes and structural brain data between the PME and the unexposed group. The chapter ended with a presentation of results of a correlation test that aimed to determine whether alterations in cortical thickness, which was observed among PME children, was associated with poorer performance in the Beery VMI test and the GPT.

CHAPTER 5

DISCUSSION

The aim of the study was to explore the effects of PME on motor development in children 8-9 years compared to unexposed children. In order to explore this, brain areas associated with motor function and cognitive tests used to evaluate motor function were used. To my knowledge, this is the first South African study that explored the effect of PME on children between the ages of 8-9 years old. Overall, the findings from this study demonstrated that PME influences cognitive development in, and brain areas associated with visual motor integration and fine motor function amongst children in this age group. In this chapter I will discuss these results within the context of available literature.

5.1. Visual-motor integration and coordination amongst PME children

This study aimed to determine if PME children will be impaired on measures of motor function, visual-motor integration and coordination, when compared to unexposed children of the same age. The results of the Beery Visual-Motor Integration test (VMI) and the Grooved Pegboard Test (GPT) demonstrated that PME children indeed performed poorer on measures of motor function, visual-motor integration and coordination. These findings are discussed in further detail below.

5.1.1. Visual-motor integration

As mentioned previously, visual-motor integration refers to the ability to coordinate fine motor skills with visual-spatial perception (Haith & Benson, 2008), and is essential for development in formal learning activities. Children between the ages of 8-9 years (i.e. during

middle childhood) are expected to be able to achieve a certain level of integration between visual and motor systems, amongst other developmental milestones.

Data from this study suggests that overall, compared to unexposed children, PME children in this study did not perform significantly worse on a measure of visual-motor integration. These findings are inconsistent with previous studies (Cernerud et al., 1996; Chang et al., 2004; Smith et al., 2011; Wouldes et al., 2014). For example, previous work by Chang et al. found that PME children performed significantly poorer in the Beery VMI test overall (Chang et al., 2004; Chang et al., 2009). However, consistent with the findings of Chang et al. (2004), PME children performed significantly worse in the Beery VMI test motor coordination sub-test.

In the Beery VMI visual perception sub-test, no significant difference was detected between groups. This finding is inconsistent with the study by Chang et al. (2004) who found visual perception impairments in PME children between the ages of 3 and 16 years. However, the IDEAL study found that PME associated visual-motor impairments seemed to resolve over time. The authors assessed the effect of PME on motor and cognitive development at ages 1 to 3 years. At the age of 1 year, PME children experienced visual-motor impairments, however, at the age of 3 years PME children presented no PME related visual-motor dysfunction (Smith et al., 2011). Inconsistencies between studies likely reflect age differences of cohorts, which emphasise the need to consider separately, specific age ranges on the developmental trajectory.

Since the Beery VMI motor coordination sub-test only assesses the motor contribution to visual-motor integration, one can speculate that PME affects the motor coordination aspect more than it affects the visual perception aspect. These results are partially consistent with a previous study that assessed the effect of PME on cognitive

functioning in children between the age 6-7 in the same cohort (Kwiatkowski et al., 2017). The findings indicated that PME children performed significantly poorer on each Beery subtest. Visual perception abilities may have improved over time due to learning so that no group effect is detectable. Impairment in the motor sub-test is consistent with evidence that suggests that motor regions of the brain are primarily targeted by PME including the striatum with lasting effects. The results suggest that PME significantly affects motor coordination skills, with moderate effects on visual-spatial perception and visual-motor integration during middle childhood.

5.1.2. Fine motor development and visual-motor coordination

Findings from the GPT demonstrated that compared to unexposed children, PME children had poorer fine motor development and visual-motor coordination. Specifically, children in the PME group performed significantly worse, as expected, in the 'insertion time with the non-dominant hand' item. Further, there was a trend for poorer performance in the PME group in 'insertion time with dominant hand', compared to the unexposed group. These findings are consistent with the findings of previous studies that demonstrated a significant difference between PME and unexposed groups in fine motor development and visual-motor coordination using similar tests (Chang et al., 2004; Smith et al., 2011; Wouldes et al., 2014). For example, in the study by Smith et al. (2011), performed in the USA, the authors used the Peabody Development Motor Scale and found impaired motor performance in PME infants between the ages of 1-2 years. Similar results were obtained amongst PME children in NZ, between the ages of 3-16 years, compared to unexposed children (Wouldes et al., 2014).

5.2. Associations of brain volume and cortical thickness findings with motor function

This study also aimed to determine if that PME children would show differences in brain volume and cortical thickness of regions underlying sensory-motor function when compared to unexposed children. It was investigated whether a correlation exists between the structural brain data and the results of the Beery VMI test and the GPT. The findings are discussed in further detail below.

5.2.1. Brain structures relating to visual-motor integration as assessed by the Beery VMI test

As discussed above, PME children performed poorer than unexposed children in visual motor integration as assessed by the Beery VMI test total score. One would expect alterations in brain structures underlying these functions. Areas that showed significant group differences and that were associated with visual-motor integration are: the caudal middle-frontal gyrus, rostral middle-frontal gyrus, parahippocampal gyrus, superior parietal gyrus, and the cuneus.

In the frontal cortex, a significant difference in cortical thickness was detected between groups in the right caudal middle-frontal gyrus and the right rostral middle-frontal gyrus and this was associated with poorer performance in the Beery VMI total score. Both areas are important in visual-motor integration. A study by Sowell et al. (2010) found alterations in the anterior cingulate cortices in PME children compared to unexposed children. The anterior cingulate cortex forms part of the attentional network, which is associated with the monitoring of control, decision making and the connection of sensory input with executive brain centres in generating motor output (Chang et al., 2004). The frontal cortex

and attentional/ executive system is still in early development during middle childhood and will show rapid development during adolescence (Sowell et al., 2004). My findings suggest aberrant frontal development in PME children that may hinder optimal integration of sensory-motor skills in later childhood.

A decrease in cortical thickness of both the left and right parahippocampal gyrus, in PME children, was observed when compared to unexposed children. The parahippocampal gyrus, a structure in the temporal lobe, is involved with visual-motor integration (Baglio et al., 2014). The decrease in cortical thickness of the parahippocampal gyrus in PME children was significantly associated with poorer performance on the Beery VMI total score. The parahippocampal cortex is involved in visuo-spatial processing and episodic memory, functions that play a fundamental role in visual-motor integration (Aminoff, Kveraga, & Bar, 2013; Haith & Benson, 2008). A study by Chang et al. (2004) found reduced volume in the hippocampal area in PME children, an area adjacent to the parahippocampal. Alterations in parahippocampal thickness may be due to aberrant neural pruning in PME children, and likely reflect suboptimal development of visual-motor integration.

Another structure that is associated with visual-motor integration, found in the parietal lobe, is the superior parietal gyrus. The superior frontal gyrus is involved with spatial orientation, receiving visual input, and sensory input (Vandenberghe, Gitelman, Parrish, & Mesulam, 2001), all functions that are crucial for visual-motor integration. PME children showed a significantly reduced left superior parietal thickness when compared with unexposed children. Similar results were observed by Roos et al. (2014), who found a reduction in cortical thickness although in inferior parietal areas in PME children. The parietal structures play a fundamental role in the integration of information from different sensory modalities, as well as integration of information that is stored in memory with

information from the sensory world (Banich & Compton, 2011). Thus, my findings are consistent with previous work that shows abnormal development of the superior parietal cortex in PME that is involved in visual-motor integration.

Lastly, significantly lower levels of cortical thickness were observed in the left cuneus in PME children when compared to unexposed children. This finding is consistent with previous work in the same cohort at the age of 6 years old and this was associated with poorer performance in sensory-motor function and visual processing (Kwiatkowski, Roos, Stein, Thomas, & Donald, 2014). Cortical thickness in the precuneus was decreased in PME children compared to unexposed children (Plomp, Leeuwen, & Ioannides, 2010; Roos et al., 2014). Studies have shown that the precuneus is involved in various cognitive processes, including visuo-spatial imagery, self-processing operations and episodic memory retrieval (Cavanna & Trimble, 2006). When considering the encompassing involvement of the cuneus in sensory-motor processes it is likely that alterations in this structure may impair the ability of PME children to adequately process and perform tasks that have components requiring visuo-spatial and motor integration.

5.2.2. Brain structures relating to fine motor development and visual-motor coordination as assessed by the Grooved Pegboard Test

As discussed above, PME children performed significantly poorer compared to unexposed children in tasks requiring fine motor development and visual-motor coordination as assessed by the GPT. Areas that showed significant group differences and that were associated with fine motor performance and visual-motor coordination are: the superior frontal gyrus and middle temporal gyrus.

A significant difference was detected in the cortical thickness of both the left and right superior frontal gyrus, an area located in the frontal cortex. As expected, a few significant differences were detected between groups in frontal structures. The frontal lobe is involved directly and indirectly with a wide range of human functions, including simple motor functions, complex motor functions and automatic motor skills (Scott & Schoenberg, 2010). These findings are consistent with previous studies that found developmental impairments in frontal structures in PME children. A study by Sowell et al. (2010) found decreased volume in the inferior frontal gyrus in PME children. Similar results were obtained by Roos et al. (2014), who found reduced cortical thickness in the inferior frontal gyrus.

Another area that showed significantly lower levels of cortical thickness, in PME children, was the left middle temporal gyrus, an area located in the temporal cortex. The middle temporal gyrus is involved in language processing and semantic memory processing, visual perception, and multimodal sensory integration (Onitsuka et al., 2004) all functions that is fundamental in visual-motor coordination and fine motor tasks. Sowell et al. (2010) also found structural alteration in temporal structures when investigating the effect of PME on brain development including: volumetric increases in middle temporal structures. Visual perception and integrating information from different sensory modalities is important in performing complex visual motor coordination, as measured by the GPT, both functions in which the middle temporal gyrus is involved (Onitsuka et al., 2004). Therefore, considering the role of the middle temporal gyrus, a reduction in cortical thickness in this area can explain weaker performance in the GPT.

Limitations and Recommendations

This study had a number of limitations. Although methamphetamine was documented to be the primary drug of abuse by mothers during pregnancy, it was difficult to validate information based on self-reported substance abuse history. Exact information on PME histories and dosages was generally unavailable, which is often the nature of this type of research (Kaltenbach & Finnegan, 1993). Although questionnaires relating to MA use during pregnancy, asked about frequency of use, this information was generally unavailable, and data were limited to 'yes/no' responses. This limitation is not uncommon in retrospective studies on prenatal drug exposure due to the difficulty of recalling a detailed drug history years after use. Many of the participants were in the care of family members or foster care parents; therefore the biological mother was not available in some instances. However, every effort was made to have telephonic contact with the mother to also verify information. Also, mothers may have been hesitant to admit to illicit drug use during pregnancy due to the stigma attached to it (Kaltenbach & Finnegan, 1993).

The second limitation is the use of polysubstances. Although some mothers also used other substances, which might have contributed to the observed effects, this was controlled for in the analysis. Polysubstance use is not uncommon in those who use MA (Gatch, Flores, & Forster, 2008). Further studies are needed in larger samples to tease out the effects of prenatal exposure to MA and other substances.

Thirdly, one should bear in mind the adverse effect of poverty on motor development when exploring the results of this study, since brain development and PME effects may be compounded by the environmental context in which these particular children reside. Participants were recruited from a school located in a community where poverty, crime and unemployment rates are extremely high. The majority of the children came from low-income

households, with an average household income of R10 000 – R20 000 per year. The adverse effects of poverty on early child development (cognitive as well as neurodevelopment) are well known (Barnett, 1998; Bellows et al., 2017; Engle & Black, 2008). A recent study in the USA, by Bellows et al. (2017), assessed the effect of poverty on motor development. They found that even though motor development is inherently established during early childhood, different environmental factors also influence the process of motor development. They found that in children from low-income households motor development was below expected norms (Bellows et al., 2017).

Longitudinal studies are needed to evaluate the exact trajectories of change in brain volumes and cortical thickness over time in children exposed to methamphetamine prenatally, as well as assessing their potential neuropsychological and neurodevelopment impact.

Other limitations of this study relate to a lack of statistical power. The ability to control for potential confounding variables are limited by the small sample size. However, considering the well-documented neurotoxic effect of MA in prenatal animal models and limited evidence in children, PME is most likely the cause of the between group differences that was observed.

Conclusion

Findings from this study have contributed to, and are mostly consistent with, previous studies on the effect of PME on early to middle childhood development. The findings of this study give us insight into how PME affects the neurodevelopment and motor function in children between the ages of 8-9 years old. Even though the mechanism underlying neurotoxicity of PME on the developing brain requires further investigation, it appears that

MA exerts its effect by altering dopaminergic, serotonergic and glutamatergic neurotransmitter systems and neuronal growth during development (Wouldes et al., 2014). The findings of this study showed that PME adversely affects fine motor skills and motor coordination, with moderate impairments in visual-spatial perception and visual-motor integration. Furthermore, lower levels of cortical thickness were found in brain areas associated with motor function in PME children. Overall, there are not enough studies on the effect of PME on neurodevelopment and cognitive functioning in humans, to draw a conclusion as to which brain is most affected by PME. However, motor function and associated brain areas seem to be especially vulnerable to the adverse effects of PME. Even though some findings from this study are partly consistent with previous work on PME, it calls for further investigation considering the present evidence that PME causes deficits in motor function and associated brain areas. Longitudinal studies that follow PME children from early to late childhood, measuring a wide spectrum of cognitive functions, might explain the neuropsychological developmental trajectory in PME children. Overall, the results of this study suggest that PME has an effect on motor performance and neurodevelopment in a sample of South African PME children. Further investigations are crucial in order to confirm the findings of the effect of PME on childhood development.

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Appendix A

Socio-demographic Questionnaire

TIK exposed children: Phase 2Background Questionnaire

Phase2 Child Participant ID: _ _ _ _ _ _ _ _ _ _ Date: _ _ / _ _ / _ _ _ _
DD / MM / YYYY

CRF Completed by: _____

Background questionnaire		
Mother / Principal caregiver details		
1	Name of primary caregiver:	
2	Relationship of primary caregiver to child e.g. grandmother:	
3	Name of mother:	
4	Person answering questionnaire:	
5	Address of child:	
6	Contact details:	a. Home no: _____ b. Work no: _____ c. Cell:_____
7	Is the mother currently employed:	☐ Yes ☐ No
	If YES: Name her occupation: How long has she been at this job:	_____

	Is she the "breadwinner": How many hours does she work a week (tick one)?	_____DD _____ MM _____ YY ☐ Yes ☐ No ☐ 20 to 40 hours / week ☐ 40 to 60 hours / week ☐ 60 to 80 hours / week
9	Marital status (tick one):	☐ Single ☐ Married ☐ Living with partner ☐ Divorced ☐ Separated ☐ Widowed
10	Household income *per year* of household where the child lives (tick one):	☐ <R10 000 ☐ R10 000 - R20 000 ☐ R 20 000 - R40 000 ☐ R40 000 - R60 000 ☐ R60 000 - R100 000 ☐ >R100 000

Child details		
Demographics		
11	Full name:	
12	Date of birth:	_ _ / _ _ _ / _ _ _ _

		DD / MMM / YYYY
13	Age:	_____Y _____M
14	Gender:	☐ Male ☐ Female
15	Level of education (grade):	
16	How long in school:	a. Preschool: _____Y_____M b. Primary school:_____Y_____M c.Total years _____
Anthropometrics (without shoes or thick clothing)		
17	Weight:	_____g
18	Height:	_____cm
19	Head circumference (HC):	_____cm
20	Upper arm circumference (UAC):	_____cm

Appendix B

Drug Intake Questionnaire

METHKI: Methamphetamine and Alcohol Exposure Questionnaire / Smoking

Visit:Neuropsychology Child Participant ID: _ _ _ _ _ _ _ _ _ _ Date: _ _ / _ _ _ / _ _ _ _
DD / MMM / YYYY

CRF Completed by: _____

Methamphetamine and Alcohol Exposure Questionnaire		
42	Person answering questionnaire:	
43	Contact details of **mother**: (Preferably, the mother should answer the questions that follow i.e. Questions 44-65.)	a. Home no: _____ b. Work no: _____ c. Cell: _____
44	How many months was the mother pregnant (according to the clinic records), when she first found out that she was pregnant?	a. _____ months b. _____ weeks
METHAMPHETAMINE EXPOSURE		
FIRST TRIMESTER (0-12 weeks) *Tick the most appropriate answer.*		
45	Did you use any methamphetamine? (If "yes", proceed to Question 46. If "no", skip Question46; continue with Question 47.)	☐ Yes ☐ No
46	If yes, how many times did you use methamphetamine per week?	☐ Once per week or less ☐ Two to three times per week ☐ Four to six times per week ☐ Daily

SECOND TRIMESTER (13-24 weeks) *Tick the most appropriate answer.*		
47	Did you use any methamphetamine? (If "yes", proceed to Question 48. If "no", skip Question 48; continue with Question 49.)	☐ Yes ☐ No
48	If yes, how many times did you use methamphetamine per week?	☐ Once per week or less ☐ Two to three times per week ☐ Four to six times per week ☐ Daily
THIRD TRIMESTER (24-40 weeks) *Tick the most appropriate answer.*		
49	Did you use any methamphetamine? (If "yes", proceed to Question 50. If "no", skip Question 50; continue with Question 51.)	☐ Yes ☐ No
50	If yes, how many times did you use methamphetamine per week?	☐ Once per week or less ☐ Two to three times per week ☐ Four to six times per week ☐ Daily

ALCOHOL EXPOSURE		
FIRST TRIMESTER (0-12 weeks) *Tick the most appropriate answer.*		
51	Did you drink any alcohol?	

	(If "yes", proceed to Question 52. If "no", skip Questions 52-53; continue with Question 54.)	☐ Yes ☐ No
52	If yes, how many times did you drink per week?	☐ Once per week or less ☐ Two to three times per week ☐ Four to six times per week ☐ Daily
53	How many drinks did you have per episode?	☐ < 2 ☐ 2 to 3 ☐ 4 or more If > 4, please specify average number:_____
SECOND TRIMESTER (13-24 weeks) *Tick the most appropriate answer.*		
54	Did you drink any alcohol? (If "yes", proceed to Question 55. If "no", skip Questions 55-56; continue with Question 57.)	☐ Yes ☐ No
55	If yes, how many times did you drink per week?	☐ Once per week or less ☐ Two to three times per week ☐ Four to six times per week ☐ Daily
56	How many drinks did you have per episode?	

		☐ < 2 ☐ 2 to 3 ☐ 4 or more If > 4, please specify average number:_____

THIRD TRIMESTER (24-40 weeks) *Tick the most appropriate answer.*		
57	Did you drink any alcohol? (If "yes", proceed to Question 58. If "no", skip Questions 58-59; continue with Question 60.)	☐ Yes ☐ No
58	If yes, how many times did you drink per week?	☐ Once per week or less ☐ Two to three times per week ☐ Four to six times per week ☐ Daily
59	How many drinks did you have per episode?	☐ < 2 ☐ 2 to 3 ☐ 4 or more If > 4, please specify average number:_____

SMOKING		
FIRST TRIMESTER (0-12 weeks) *Tick the most appropriate answer.*		
60	Did you smoke? (If "yes", proceed to Question 61. If "no", skip Question 61; continue with Question 62.)	☐ Yes ☐ No
61	If yes, how many cigarettes did you smoke per day?	☐ 10 or less ☐ 11 to 20 ☐ 21 to 30 ☐ More than 30
SECOND TRIMESTER (13-24 weeks) *Tick the most appropriate answer.*		
62	Did you smoke? (If "yes", proceed to Question 63. If "no", skip Question 63; continue with Question 64.)	☐ Yes ☐ No
63	If yes, how many cigarettes did you smoke per day?	☐ 10 or less ☐ 11 to 20 ☐ 21 to 30 ☐ More than 30
THIRD TRIMESTER (24-40 weeks) *Tick the most appropriate answer.*		
64	Did you smoke? (If "yes", proceed to Question 65.	☐ Yes

	If “no”, skip Question 65.)	☐ No
65	If yes, how many cigarettes did you smoke per day?	☐ 10 or less ☐ 11 to 20 ☐ 21 to 30 ☐ More than 30

Appendix C

Ethics Approval: Letter of Confirmation

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Approval Notice
New Application

06-Sep-2016
Du Toit, Stefani S

Proposal #: SU-HSD-002904
Title: Structural Brain Differences and Motor functioning in Prenatally Methamphetamine Exposed Children in Cape Town

Dear Miss Stefani Du Toit,

Your **New Application** received on **08-Aug-2016**, was reviewed
Please note the following information about your approved research proposal:

Proposal Approval Period: **06-Sep-2016 -05-Sep-2019**

General comments:
Please note that the following comment is a recommendation from the REC reviewer in his/her personal capacity to further enhance the benefit of this valuable study. No response is therefore required from the applicant:
"I would like to add that the result (whatever it may yield) should be disseminated appropriately to the parents (if this was the case in the larger study)"

Please take note of the general Investigator Responsibilities attached to this letter. You may commence with your research after complying fully with these guidelines.

Please remember to use your **proposal number** (**SU-HSD-002904**) on any documents or correspondence with the REC concerning your research proposal.

Please note that the REC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Also note that a progress report should be submitted to the Committee before the approval period has expired if a continuation is required. The Committee will then consider the continuation of the project for a further year (if necessary).

This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki and the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health). Annually a number of projects may be selected randomly for an external audit.

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637: IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30/05/2016
☐ Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	

Comments to PI from the HREC

HUMAN RESEARCH ETHICS COMMITTEE
15 OCT 2015
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	13/10/2015		
HREC REF Number	235/2009	Current Ethics Approval was granted until	May 2015
Protocol title	Structural neuro-imaging and neuro-cognitive correlates in prenatally methamphetamine exposed children in Cape Town		
Protocol number (if applicable)	HREC 235/2009		
Are there any sub-studies linked to this study?	☐ Yes ✓ No		
If yes, could you please provide the HREC Ref's for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.	n/a		
Principal Investigator	Kirsty Donald		
Department / Office Internal Mail Address	Department of Paediatrics		

1.1 Does this protocol receive US Federal funding?	☐ Yes	✓ No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval?	☐ Yes	☐ No n/a
1.3 Has sponsorship of this study changed? If yes, please attach a revised summary of the budget.	☐ Yes	✓ No

Appendix D

Informed Consent form

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM FOR USE BY PARENTS/LEGAL GUARDIANS Children Control Version

TITLE OF THE RESEARCH PROJECT:

STRUCTURAL NEURO-IMAGING AND NEURO-COGNITIVE CORRELATES IN PRENATALLY METHAMPHETAMINE EXPOSED CHILDREN IN CAPE TOWN.

REFERENCE NUMBER: HREC 235/2009

PRINCIPAL INVESTIGATOR: Dr Kirsty Donald

ADDRESS: School of Child and Adolescent Health, Red Cross Children's Hospital and the University of Cape Town

CONTACT NUMBER: (021) 6585322

Your child is being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how your child could be involved. Also, your child's participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you or your child negatively in any way whatsoever. You are also free to withdraw him/her from the study at any point, even if you do initially agree to let him/her take part.

This study has been approved by the Committee for Human Research at the University of Cape Town and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

This study looks at the structure of your child's brain using a brain scan (Magnetic resonance imaging) and by doing some tests of learning. By doing so we hope to get a better understanding of how the brain looks and also what goes wrong in certain disorders so (in the long term) we can identify problems and develop better treatments.

Why has your child been invited to participate?
Previously we tested your child's development and we would now like to look at the structure of his/her brain as well as to do some further tests of learning and behaviour.

What will your responsibilities be?
You would be required to bring your child to the unit so we can get the images and wait with your child while we are scanning. Your child will also be given an assessment of learning on a different day at the Red Cross Children's Hospital. Each session will take 1-2 hours in total. There will be one set of assessments this year and another (similar) set in a year's time.

Will your child benefit from taking part in this research?
An assessment of your child's learning will be done and brain scanned. Possible problems may be picked up early and your child will be referred for treatment.

Are there any risks involved in your child taking part in this research?
No. Your child may become bored and not find this enjoyable but they will experience no pain. If at any time they become upset and do not wish to continue, the task will be stopped.

Who will have access to your child's medical records?
Only members of the research team will have access to the data gathered here. All information will remain confidential and if the results of this study are published no participant will be identified.

Will you or your child be paid to take part in this study and are there any costs involved?
You or your child will not be paid to take part in the study, but your/your child's transport and meal costs will be covered for each study visit. There will be no costs involved for you if your child does take part.

Is there any thing else that you should know or do?

- You should inform your family practitioner or usual doctor that your child is taking part in a research study.
- You can contact Dr Kirsty Donald at tel 021-6585322 if you have any further queries or encounter any problems.
- You can contact the Committee for Human Research if you have any concerns or complaints that have not been adequately addressed by your child's study doctor.

- You will receive a copy of this information and consent form for your own records.

Assent of minor

I (*Name of Child/Minor*)…… have been invited to take part in the above research project.

- The study doctor/nurse and my parents have explained the details of the study to me and I understand what they have said to me.
- They have also explained that this study will involve. I also know that I am free to withdraw from the study at any time if I am unhappy.
- By writing my name below, I voluntary agree to take part in this research project. I confirm that I have not been forced either by my parents or doctor to take part.

…… ……

Name of child **Independent witness**

(To be written by the child if possible)

Declaration by parent/legal guardian

By signing below, I (*name of parent/legal guardian*) ……...

agree to allow my child (name of child……who is…….

years old, to take part in a research study entitled (insert title of the study)

I declare that:

- I have read or had read to me this information and consent form and that it is written in a language with which I am fluent and comfortable.
- If my child is older then 7 years, he/she must agree to take part in the study and his/her ASSENT must be recorded on this form.
- I have had a chance to ask questions and all my questions have been adequately answered.

- I understand that taking part in this study is **voluntary** and I have not been pressurised to let my child take part.
- I may choose to withdraw my child from the study at any time and my child will not be penalised or prejudiced in any way.
- My child may be asked to leave the study before it has finished if the study doctor or researcher feels it is in my child's best interests, or if my child does not follow the study plan as agreed to.

Signed at (*place*) .....…….. on (*date*) ……..…….. 2013.

....

Signature of parent/legal guardian **Signature of witness**

Declaration by investigator

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understand all aspects of the research, as discussed above
- I did/did not use a translator (i*f a translator is used, then the translator must sign the declaration below).*

Signed at (*place*) .....…….. on (*date*) ……..…….. 2013.

....

Signature of investigator **Signature of witness**

Declaration by translator

I *(name)* ……..…… declare that:

- I assisted the investigator (*name*) ……..…….. to explain the information in this document to (*name of parent/legal guardian*) ……..……... using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the parent/legal guardian fully understands the content of this informed consent document and has had all his/her questions satisfactorily answered.

Signed at (*place*) .....…….. on (*date*) ……..…….. 2013.

....

Signature of translator **Signature of witness**

Appendix E

Informed Assent Form

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM FOR USE BY PARENTS/LEGAL GUARDIANS Children Patient Version

TITLE OF THE RESEARCH PROJECT:

STRUCTURAL NEURO-IMAGING AND NEURO-COGNITIVE CORRELATES IN PRENATALLY METHAMPHETAMINE EXPOSED CHILDREN IN CAPE TOWN.

REFERENCE NUMBER: HREC 235/2009

PRINCIPAL INVESTIGATOR: Dr Kirsty Donald

ADDRESS: School of Child and Adolescent Health, Red Cross Children's Hospital and the University of Cape Town

CONTACT NUMBER: (021) 6585322

Your child is being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how your child could be involved. Also, your child's participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you or your child negatively in any way whatsoever. You are also free to withdraw him/her from the study at any point, even if you do initially agree to let him/her take part.

This study has been approved by the Committee for Human Research at University of Cape Town and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

This study looks at the structure of your child's brain using a brain scan (Magnetic resonance imaging) and by doing some tests of learning and behaviour**.** By doing so we hope to get a better understanding of how the brain looks as well as how a child learns and also what goes wrong in certain disorders so (in the long term) we can identify problems and develop better treatments.

Why has your child been invited to participate?
We would like to get a better understanding of brain structure as well as the way children learn and behave who have been exposed to a substance such as Methamphetamine ('Tik'), with the aim of eventually improving diagnosis and treatment options.

What will your responsibilities be?
You would be required to bring your child to the unit so we can get the images and wait with your child while we are scanning. Your child will also be given an assessment of learning on a different day at the Red Cross Children's Hospital. Each session will take 1-2 hours in total. There will be one set of assessments this year and another (similar) set in a year's time.

Will your child benefit from taking part in this research?
Your child will receive a new type of brain scan as well as a report from the tests of learning. Any information obtained will be sent to your child's doctor (with your consent). This may or may not aid in your child's treatment.

Are there any risks involved in your child taking part in this research?
No. Your child may become bored and not find this enjoyable but they will experience no pain. If at any time they become upset and do not wish to continue, the task will be stopped.

Who will have access to your child's medical records?
Only members of the research team will have access to the data gathered here. All information will remain confidential and if the results of this study are published no participant will be identified. We may require access to your child's medical records. We will only ask for access to these records with your written permission.

Will you or your child be paid to take part in this study and are there any costs involved?
You or your child will not be paid to take part in the study, but your/your child's transport and meal costs will be covered for each study visit. There will be no costs involved for you if your child does take part.

Is there any thing else that you should know or do?

- You should inform your family practitioner or usual doctor that your child is taking part in a research study.
- You can contact Dr Kirsty Donald tel 6585322 if you have any further queries or encounter any problems.
- You can contact the Committee for Human Research at if you have any concerns or complaints that have not been adequately addressed by your child's study doctor.
- You will receive a copy of this information and consent form for your own records.

Assent of minor

I (*Name of Child/Minor*)……. have been invited to take part in the above research project.

- The study doctor/nurse and my parents have explained the details of the study to me and I understand what they have said to me.

- They have also explained that this study will involve. I also know that I am free to withdraw from the study at any time if I am unhappy.
- By writing my name below, I voluntary agree to take part in this research project. I confirm that I have not been forced either by my parents or doctor to take part.

....

Name of child **Independent witness**

(To be written by the child if possible)

Declaration by parent/legal guardian

By signing below, I *(name of parent/legal guardian)* ……..…….

agree to allow my child (name of child) ……..… who is …….

years old, to take part in a research study entitled *(insert title of study)*

I declare that:

- I have read or had read to me this information and consent form and that it is written in a language with which I am fluent and comfortable.
- If my child is older then 7 years, he/she must agree to take part in the study and his/her ASSENT must be recorded on this form.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to let my child take part.
- I may choose to withdraw my child from the study at any time and my child will not be penalised or prejudiced in any way.
- My child may be asked to leave the study before it has finished if the study doctor or researcher feels it is in my child's best interests, or if my child does not follow the study plan as agreed to.

Signed at (*place*) .....…..…….. on (*date*) ……...…….. 2013.

.....	.....
Signature of parent/legal guardian	**Signature of witness**

Declaration by investigator

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understand all aspects of the research, as discussed above
- I did/did not use a translator (i*f a translator is used, then the translator must sign the declaration below).*

Signed at (*place*) .....…..…….. on (*date*) ……...…….. 2013.

.....	.....
Signature of investigator	Signature of witness

Declaration by translator

I *(name)* ……..…… declare that:

- I assisted the investigator (*name*) ……..……. to explain the information in this document to (*name of parent/legal guardian*) ……...……... using the language medium of Afrikaans/Xhosa.

- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the parent/legal guardian fully understands the content of this informed consent document and has had all his/her questions satisfactorily answered.

Signed at (*place*) .....…..…….. on (*date*) ……...…….. 2013.

…… ……

Signature of translator **Signature of witness**

I declare that:

I grant/do not grant the researcher permission to make my child's results known to my treating doctor

Signed at (*place*)……..on (*date*) …….. 2013

…….. ……

Signature of Participant **Signature of Witness.**

I declare that:

I grant/do not grant the researcher permission to access my child's medical records.

Signed at (*place*)……..on (*date*) …….. 2013

…….. ……

Signature of Participant **Signature of Witness.**

Appendix F

T-test/ Mann-Whitney U-test Results for Brain Volumes and Cortical Thicknesses

Brain Area	Group		*t / U*	*df*	*p*
	PME (n = 17)	UNEXPOSED (n = 16)			
L – Lateral Ventricle	.0033 (.0021)	.0027 (.0006)	-.992	31	.33
L – Inferior Lateral Ventricle	.0002 (.0002)	.0002 (.0001)	-.362	31	.72
L – Cerebellum WM	.0097 (.0011)	.0093 (.0009)	-1.25	31	.22
L - Cerebellum Cortex	.0426 (.0045)	.0408 (.0041)	-1.23	31	.23
L – Thalamus Proper	.0053 (.0004)	.0054 (.004)	.60	31	.55
L – Caudate	.0030 (.0004)	.0030 (.0003)	.15	31	.89
L - Putamen[A]	.0052 (.0006)	.0049 (.0003)	99	31	.19
L – Pallidum[A]	.0016 (.0002)	.0015 (.0001)	135	31	.99
Brain Stem	.0141 (.0018)	.0147 (.0012)	1.20	31	.24
L – Hippocampus	.0028 (.0006)	.0029 (.0002)	1.05	31	.30
L – Amygdala	.0010 (.0001)	.0011 (.0002)	1.08	31	.29
CSF	.0006 (.0002)	.0007 (.0002)	.72	31	.48
L – Accumbens area	.0006 (.0001)	.0006 (.0001)	.93	31	.36

Brain Area	Group		*t / U*	*df*	*p*
	PME (n = 17)	UNEXPOSED (n = 16)			
L – Ventral DC	.0030 (.0003)	.0029 (.0002)	-.35	31	.73
R – Lateral Ventricle	.0027 (.0017)	.0026 (.0011)	-.22	31	.82
R – Inferior Lateral Ventricle	.0002 (.0001)	.0002 (.0001)	-.77	31	.45
R – Cerebellum WM	.0096 (.0012)	.0095 (.0011)	-.14	31	.89
R – Cerebellum Cortex	.0430 (.0041)	.0410 (.0038)	-1.43	31	.16
R – Thalamus Proper	.0054 (.0003)	.0055 (.0005)	.85	31	.41
R – Caudate	.0031 (.0003)	.0031 (.0003)	.61	31	.55
R – Putamen [A]	.0050 (.0006)	.0048 (.0003)	102	31	.23
R – Pallidum	.0014 (.0001)	.0014 (.0000)	-1.20	31	.24
R – Hippocampus	.0030 (.0002)	.0030 (.0002)	.65	31	.52
R – Amygdala	.0011 (.0001)	.0011 (.0001)	-.47	31	.64
R – Accumbens Area	.0006 (.0001)	.0006 (.0001)	-.40	31	.73
R – Ventral DC	.0030 (.0003)	.0030 (.0002)	-.50	31	.62
CC – Posterior	.0006 (.0001)	.0006 (.0001)	-.63	31	.54
CC – Mid-Posterior	.0003 (.0001)	.0003 (.0001)	.98	31	.33
CC – Central	.0004 (.0001)	.0004 (.0001)	.58	31	.57

Brain Area	Group		*t / U*	*df*	*p*
	PME (n = 17)	UNEXPOSED (n = 16)			
CC – Mid Anterior	.0005 (.0001)	.0005 (.0001)	-.66	31	.52
CC – Anterior	.0008 (.0001)	.0007 (.0001)	-.82	31	.42
LH – Cortex volume	.2058 (.0158)	.2116 (.0133)	1.13	31	.27
RH – Cortex volume	.2083 (.0157)	.2129 (.0155)	.85	31	.40
Cortex volume	.4141 (.0312)	.4245 (.0286)	.99	31	.33
LH – Cortical WM volume	.1597 (.0098)	.1606 (.0081)	.30	31	.77
RH – Cortical WM volume	.1617 (.0088)	.1627 (.0087)	.32	31	.75
Cortical WM volume	.3214 (.0184)	.3233 (.0167)	.31	31	.76
Sub-Cortical Gray volume	.1447 (.0118)	.1414 (.0097)	-.88	31	.39
Total Gray volume	.5588 (.0368)	.5658 (.0323)	.59	31	.56
Supra Tentorial volume	.8116 (.0444)	.8225 (.0389)	.75	31	.46
Intra Cranial Volume	1223 (9037)	1258 (9360)	1.07	31	.29
LH – Bankssts CT	2.773 (.2176)	2.821 (.2007)	.66	31	.52
LH – Caudal Anterior Cangulate CT	2.603 (.3033)	2.693 (.2628)	.91	31	.37
LH – Caudal Middle Frontal T[A]	2.815 (.2668)	2.914 (.1211)	94	31	.14
LH – Cuneus CT	2.284 (.2049)	2.435 (.1877)	2.21	31	.04*

Brain Area	Group		*t / U*	*df*	*p*
	PME (n = 17)	UNEXPOSED (n = 16)			
LH – Entorhinal CT	3.545 (.4774)	3.653 (.5241)	.62	31	.54
LH – Fusiform CT[A]	2.862 (.2590)	2.908 (.1281)	130	31	.85
LH – Inferior Parietal CT[A]	2.910 (.0733)	2.973 (.1340)	115	31	.47
LH – Inferioir Temporal CT	2.988 (.2687)	3.073 (.2077)	1.01	31	.32
LH – Isthmus Cingulate CT	2.848 (.2661)	2.803 (.2007)	-.548	31	.59
LH – Lateral Occipital CT[A]	2.363 (.1562)	2.445 (.0860)	95	31	.15
LH – Lateral Orbito Frontal CT	3.195 (.2624)	3.321 (.1895)	1.57	31	.13
LH – Lingual CT	2.365 (.1092)	2.413 (.1626)	.99	31	.33
LH Medial Orbito Frontal CT	2.955 (.1569)	2.982 (.1651)	.48	31	.63
LH Middle Temporal CT	3.066 (.1885)	3.209 (.1748)	2.25	31	.03*
LH – Parahippocampal CT	2.464 (.2778)	2.859 (.2455)	4.32	31	<.001***
LH – Paracentral CT	2.880 (.1655)	2.858 (.1688)	-.370	31	.71
LH – Parsopercularis CT	2.845 (.2128)	2.966 (.1623)	1.83	31	.08
LH – Parsorbitalis CT	3.405 (.2464)	3.447 (.2985)	.439	31	.66
LH – Parstriangularis CT[A]	2.970 (.2115)	3.030 (.1314)	122	31	.63
LH – Pericalcarine CT	2.094 (.2225)	2.055 (.2309)	-.497	31	.62

Brain Area	Group		*t / U*	*df*	*p*
	PME (n = 17)	UNEXPOSED (n = 16)			
LH – Postcentral CT	2.285 (.1475)	2.301 (.1374)	.334	31	.74
LH – Posterior Cingulate CT[A]	2.823 (.2752)	2.785 (.1389)	130	31	.85
LH – Precentral CT[A]	2.603 (.1612)	2.681 (.0675)	96.5	31	.16
LH – Precuneus CT	2.834 (.1268)	2.882 (.1456)	1.02	31	.32
LH – Rostral Anterioir Cingulate CT	2.934 (.2426)	3.073 (.2039)	1.78	31	.08
LH – Rostral Middle Frontal CT	2.825 (.1590)	2.913 (.1774)	1.51	31	.14
LH – Superior Frontal CT[A]	3.211 (.2032)	3.362 (.1280)	78.5	31	.04*
LH – Superior Parietal CT	2.578 (.1160)	2.670 (.1296)	2.13	31	.04*
LH – Superior Temporal CT[A]	2.966 (.2495)	3.109 (.1802)	83	31	.05*
LH – Supramarginal CT	2.865 (.1781)	2.952 (.1383)	1.56	31	.13
LH – Frontal Pole CT	3.481 (.3459)	3.585 (.3752)	.82	31	.42
LH – Temporal Pole CT	3.802 (.4559)	3.896 (.4047)	.63	31	.54
LH – Transverse Temporal CT	2.586 (.2084)	2.652 (.2553)	.82	31	.42
LH – Insula CT	3.114 (.2470)	3.165 (.1973)	.65	31	.52
RH – Bankssts CT	2.844 (.2375)	2.993 (.2158)	1.88	31	.07
RH – Caudal anterior cingulate CT [A]	2.488 (.3146)	2.506 (.1748)	132.5	31	.90

Brain Area	Group		*t / U*	*df*	*p*
	PME (n = 17)	UNEXPOSED (n = 16)			
RH – Caudal Middle Frontal CT	2.795 (.3019)	2.990 (.1852)	2.22	31	.03*
RH – Cuneus CT	2.309 (.1952)	2.366 (.1671)	.91	31	.37
RH – Entorhinal CT	3.682 (.6132)	3.809 (.4893)	.65	31	.52
RH – Fusiform CT	2.898 (.2167)	3.000 (.1446)	1.59	31	.12
RH – Inferior Parietal CT	2.928 (.1422)	2.998 (.1319)	1.47	31	.15
RH – Inferior Temporal CT	3.051 (.3026)	3.102 (.2797)	.50	31	.62
RH – Isthmuscingulate CT[A]	2.704 (.2492)	2.731 (.1782)	126	31	.74
RH – Lateral Occipital CT	2.421 (.1957)	2.467 (.1155)	.12	31	.42
RH Lateral Orbito Frontal CT	3.155 (.2627)	3.209 (.1959)	.66	31	.52
RH – Lingual CT	2.407 (.1505)	2.458 (.1337)	1.02	31	.32
RH – Medial Orbito Frontal CT	2.934 (.2369)	2.957 (.1803)	.309	31	.76
RH – Middle Temporal CT	3.120 (.2286)	3.257 (.1819)	1.90	31	.07
RH – Parahippocampus CT	2.581 (.3099)	2.760 (1560)	2.07	31	.05*
RH – Paracentral CT[A]	2.771 (.2686)	2.846 (.1387)	95	31	.15
RH – Parsopercularis CT	2.885 (.2044)	3.008 (.1894)	1.79	31	.08
RH – Parsorbitalis CT	3.365 (.2765)	3.447 (.2059)	.97	31	.34

Brain Area	Group		*t / U*	*df*	*p*
	PME (n = 17)	UNEXPOSED (n = 16)			
RH- Parstriangularis CT	3.026 (.1908)	3.040 (.1813)	.20	31	.84
RH – Pericalcarine CT[A]	2.037 (.2984)	1.997 (.1643)	134	31	.96
RH – Postcentral CT	2.313 (.1768)	2.284 (.1157)	-.56	31	.58
RH – Posterior Cingualate CT	2.658 (.2449)	2.670 (.1565)	.17	31	.87
RH – Precentral CT[A]	2.588 (.2417)	2.701 (.1086)	104	31	.26
RH – Precuneus CT	2.798 (.1492)	2.869 (.1265)	1.47	31	.15
RH – Rostral Anterior Cingulate CT	2.825 (.2326)	2.937 (.2715)	1.27	31	.22
RH – Rostral Middle Frontal CT	2.801 (.1579)	2.920 (.1457)	2.24	31	.03*
RH – Superior Frontal CT	3.166 (.1864)	3.306 (.1839)	2.16	31	.04*
RH- Superior Parietal CT	2.548 (.1345)	2.635(.1343)	1.86	31	.07
RH – Superior Temporal CT	3.050 (.2110)	3.160 (.1400)	1.75	31	.09
RH – Supramarginal CT	2.883 (.2192)	2.965 (.1335)	1.28	31	.21
RH – Frontal Pole CT	3.390 (.3376)	3.495 (.3516)	.88	31	.39
RH – Temporal Pole CT	3.848 (.5296)	3.890 (.2881)	.28	31	.78
RH – Transversetemporal CT	2.631 (.2660)	2.753 (.2456)	1.36	31	.18
RH – Insula CT	3.067 (.2552)	3.123 (.1812)	.72	31	.48

Note. Data presented are p-values. PME = prenatal methamphetamine exposed; CT = Cortical Thickness; WM = White Matter

[A]Assumption of normality was not upheld, Mann-Whitney *U* test was used.